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RHAPONTICIN, AND ANTHRAQUINONE DERIVATIVES FROM *RHEUM EMODI*, WALL. (INDIAN, OR HIMALAYAN RHUBARB).

BY

M. Ghouse Mohiuddin and M. C. Tummin Katti.

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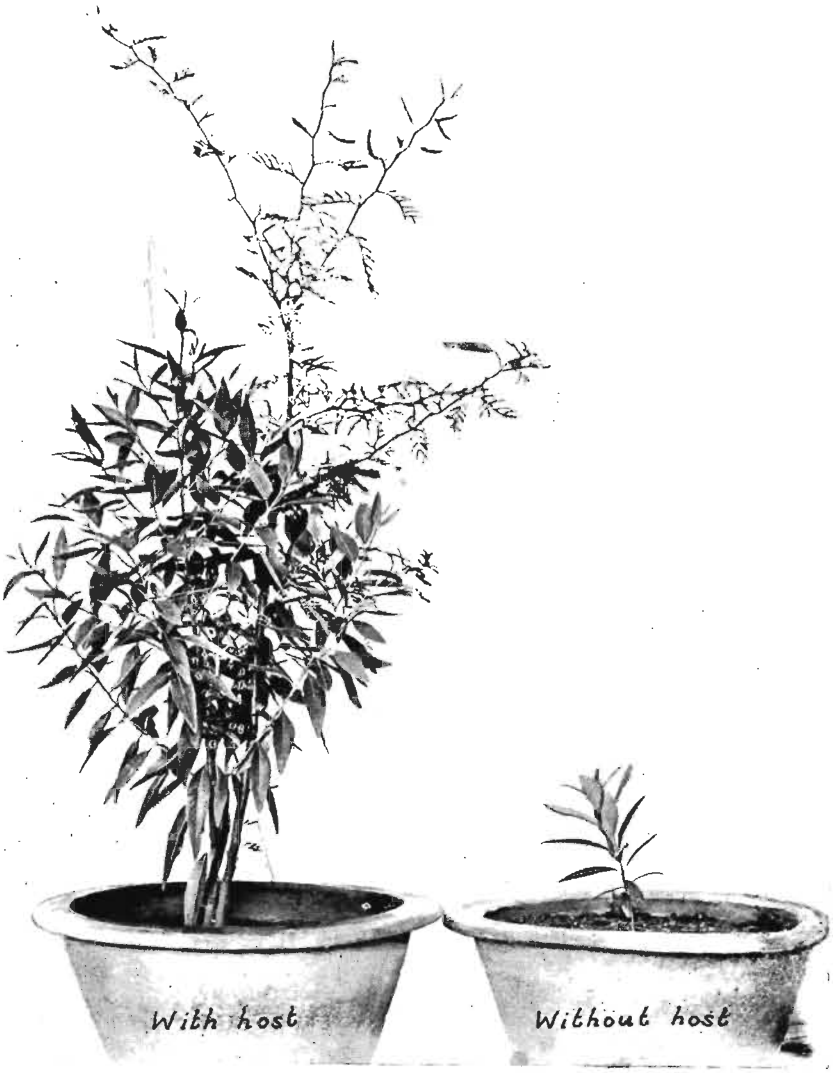
With host
Without host

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ABBREVIATIONS OF TITLES

ABBREVIATION	NAME OF PERIODICAL
Agric. J. India	Agricultural Journal of India.
Agric. Res. Inst. Pusa	Agricultural Research Institute, Pusa.
Amer. J. Pub. Health	American Journal of Public Health.
Anal. Fis. Quim.	Anales de la Sociedad Española Física y Química.
Annalen	Justus Liebig's Annalen der Chemie.
Ann. Appl. Biol.	Annals of Applied Biology.
Ann. Chim. Applicata	Annali di Chimica Applicata.
Arch. Pharm.	Archiv der Pharmazie.
Bell System Tec. J.	Bell System Technical Journal.
Ber.	Berichte der deutschen chemischen Gesellschaft.
Biochem. J.	The Biochemical Journal.
Biochem. Zeit.	Biochemische Zeitschrift.
Bull. Imp. Inst.	Bulletin of the Imperial Institute.
Bur. Standards Bull.	Bureau of Standards Bulletin.
Bur. Stand. J. Research	Bureau of Standards Journal of Research.
Centralbl. Bakt.	Zentralblatt für Bakteriologie.
Chem. Ind.	Chemistry and Industry.
Compt. rend.	Comptes rendus hebdomadaires des Scéances de l'Academie des Sciences.
D. R. P.	Deutsches Reichspatent.
Elec. Jour.	Electric Journal.
Eng. News Rec.	Engineering News Record.
E. T. Z.	Elektrotechnische Zeitschrift.
Florida Agric. Expt. Sta. Bull.	Florida Agricultural Experimental Station Bulletin.
Helv. Chim. Acta	Helvetica Chimica Acta.
Ind. Eng. Chem.	Industrial and Engineering Chemistry.
Ind. J. Med. Res.	Indian Journal of Medical Research.
Ind. Sci. Congress, Med. Sec.	Indian Science Congress, Medical Section.
International Cong. App. Chem.	International Congress of Applied Chemistry.
J. Agric. Sci.	Journal of Agricultural Science.
J. Amer. Chem. Soc.	Journal of the American Chemical Society.
J. Amer. Soc. Agron.	Journal of the American Society of Agronomy.
J. Biol. Chem.	Journal of Biological Chemistry.
J. C. S.	Journal of the Chemical Society.

Abbreviation	Name of Periodical
J. Gen. Physiol.	Journal of General Physiology.
J. I. E. E.	Journal of the Institution of Electrical Engineers.
J. Ind. Chem. Soc.	Journal of the Indian Chemical Society.
J. Ind. Inst. Sci.	Journal of the Indian Institute of Science.
J. Inst. Elec. Eng.	Journal of the Institution of Electrical Engineers.
J. Int. Soc. L. T. C.	Journal of the International Society of Leather Trades Chemists.
J. Min. Agric.	Journal of the Ministry of Agriculture and Fisheries.
J. Pharm. Soc. Japan	Journal of the Pharamaceutical Society of Japan.
J. Phys. Chem.	Journal of Physical Chemistry.
J. Physiol.	Journal of Physiology.
J. pr. Chem.	Journal für practische Chemie.
J. S. C. I.	Journal of the Society of Chemical Industry.
J. S. C. I., Rev.	Journal of the Society of Chemical Industry, Review.
J. Soc. Chem. Ind.	Journal of the Society of Chemical Industry.
J. West. Soc. Eng.	Journal of the Western Society of Engineers.
Monatsh.	Monatshefte für Chemie und verwandte Theile anderer Wissenschaften.
Perf. and Essent. Oil. Rec.	Perfumery and Essential Oil Record.
Phys. Rev.	Physical Review.
Proc. I. R. E.	Proceedings of the Institute of Radio Engineers.
Proc. Ind. Sci. Cong.	Proceedings of the Indian Science Congress.
Proc. Roy. Soc.	Proceedings of the Royal Society.
Proc. Roy. Soc. Canada	Proceedings of the Royal Society of Canada.
Soil Sci.	Soil Science.
Zentralbl. Bakt.	Zentralblatt für Bakteriologie.
Z. pflanz. D.	Zeitschrift für Pflanzenernährung und Düngung.
Z. physikal. Chem.	Zeitschrift für physikalische Chemie, Stöchiometrie und Verwandtschaftslehre.
Z. physiol. Chem.	Hoppe-Seyler's Zeitschrift für physiologische Chemie.

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RHAPONTICIN, AND ANTHRAQUINONE DERIVATIVES FROM *RHEUM EMODI*, WALL. (INDIAN, OR HIMALAYAN RHUBARB).

By M. Ghouse Mohiuddin and M. C. Tummin Katti.

The Chinese rhubarb of commerce, which is official in the Pharmacopoeias of many European countries and the United States of America, is either *Rheum palmatum*, Linn., *Rheum officinale*, Baill., or "other species of *Rheum* grown in China and Tibet", and is one of the most popular cathartic drugs. It generally comes to the European market from China and is sometimes found adulterated with less active varieties of *Rheum*, e.g., *Rheum rhaponticum*, Linn., which are found in Austria and adjoining countries; in recent years, however, the official rhubarb has been grown in England, France and other European countries. Since it is generally known that the glucosides of hydroxyanthraquinones are at least partially responsible for the cathartic action of rhubarb, its chemistry, mainly with respect to the nature of the anthraquinone derivatives, has engaged the attention of many investigators.

Although the earliest inquiries date from the first quarter of the last century, it is only since the beginning of this century that systematic work on the drug has been conducted. Hesse (*Annalen*, 1899, **309,** 32) found chrysophanic acid and its methyl ether, emodin, rhabarberon (an isomer of emodin), rhein, and some other compounds in a sample of Chinese rhubarb (*Rheum officinale*, Baill.) and chrysophanic acid and rhaponticin in Austrian (*Rheum rhaponticum*, Linn.) and English rhubarbs. The sample of English rhubarb, however, was shown by the same author (*J. pr. Chem.*, 1908, **77,** 32) to be *Rheum rhaponticum*, Linn. and not *Rheum palmatum*, Linn. as originally supposed. In this connection he also described the analysis of Austrian rhubarb which was found to contain rhaponticin, anhydro-rhapontigenin, $C_{15}H_{12}O_3$, iso-rhapontigenin, $C_{15}H_{14}O_4$, chrysophanic acid, $C_{15}H_{10}O_4$, and two other isomers of emodin, $C_{15}H_{10}O_5$, with gallic acid, rhapontic acid, $C_{17}H_{16}O_6$, and glucosides of anthraquinone derivatives. The methyl ether of chrysophanic acid has been shown indirectly by Oesterle and Johann (*Arch. Pharm.*, 1910, **248,** 476) to be impure chrysophanic acid mixed with emodin monomethyl ether which is usually associated with the former in rhubarb.

Tschirch and Heuberger (*Arch. Pharm.*, 1902, **240**, 596) isolated from a sample of Chinese rhubarb, presumably *Rheum officinale*, Baill., chrysophanic acid, emodin, rhein and a mixture of tannin- and hydroxyanthraquinone-glucosides. Tschirch and Cristofoletti (*Arch. Pharm.*, 1905, **243**, 443) found in Austrian rhubarb rhaponticin, chrysophanic acid and its methyl ether (*cf.* Oesterle and Johann, *loc. cit.*), chrysopontin, $C_{16}H_{16}O_5$, corresponding in composition to methoxydihydroxymethyltetrahydroanthraquinone, and a mixture of hydroxyanthraquinone-glucosides. They could not isolate either emodin or rhein. Tschirch and Edner (*Arch. Pharm.*, 1907, **245**, 139) isolated from English rhubarb (*Rheum officinale*, Baill.) only chrysophanic acid, emodin, iso-emodin (rhabarberon of Hesse, *loc. cit.*) without rhaponticin ; in French rhubarb (*Rheum rhaponticum*, Linn.) they found rhaponticin, chrysophanic acid and chrysopontin.

Tutin and Clewer (*J.C.S.*, 1911, **99**, 946) found in a sample of "Shensi" (Chinese) rhubarb, cinnamic and gallic acids, rhein, emodin and its monomethyl ether, chrysophanic acid, aloe-emodin, rheinolic acid, a crystalline mixture of the glucosides of most of the above hydroxyanthraquinones, together with a non-glucosidic resin which, on hydrolysis with alkali, gave small amounts of cinnamic acid, emodin, aloe-emodin and gallic acid. They found this last non-glucosidic resin to be physiologically the most active constituent.

Tschirch and Ruszkowski (*Arch. Pharm.*, 1913, **251**, 121) found in a Russian variety of rhubarb (*Rheum rhaponticum*, Linn.) rhaponticin, emodin and its monomethyl ether, chrysophanic acid with tannin- and hyroxyanthraquinone-glucosides. Semmel (*Arch. Pharm.*, 1918, **256**, 91) found "Shensi" rhubarb (*Rheum palmatum*, Linn.) to contain chrysophanic acid, aloe-emodin, rhein, and hydroxyanthraquinone-glucosides. Holmström (*Schweiz. Apoth. Ztg.*, 1921, **59**, 169 and 183 ; through *Chem. Abs.*, 1921, **15**, 2427) during his analysis of *Rheum emodi*, Webb. (probably from Switzerland), found rhaponticin, chrysophanic acid, emodin (?) and a compound similar to rheochrysin.

Rheum emodi, Wall. belonging to the natural order, *Polygonaceae*, is one of the Indian rhubarb species found wild in Nepal, Kashmir, Sikkim and Bhutan at altitudes of eleven to twelve thousand feet. The Central Indigenous Drugs Committee (which functioned only between the years 1901 and 1916) appointed by the Government of India to make a clinical study of indigenous drugs the properties of which were already known, subjected this drug to a detailed clinical test. The Committee assumed that it contained only the anthraquinone derivatives usually found in official rhubarb, and found (Second Report, 1909, pp. 71-74 and Third Report, 1916, pp. 1-22) that the powdered drug caused

griping pain in a large number of cases ; the general opinion was that it was unsatisfactory as a purgative.

Griping action is not usually associated with preparations from official rhubarb and since no chemical examination of Indian or Himalayan rhubarb has been made hitherto, it seemed desirable to seek definite information regarding the constituents responsible for the griping effect, and to survey the anthraquinone derivatives responsible for its cathartic action.

EXPERIMENTAL.

The material employed consisted of dry and powdered rhizomes obtained from Kashmir forests.

Preliminary examination for alkaloids.—Digestion with Prollius's fluid for four hours gave negative results.

Starch, other carbohydrates and tannins.—The filtrate from thorough digestion with warm water for about half an hour showed the presence of starch, tannins, and a reducing sugar.

Steam distillation.—About one litre of acidic distillate from 500 g. was thoroughly extracted with ether, and this after washing with sodium carbonate solution followed by sodium hydroxide left on distillation a small amount of pale yellow oil having the characteristic odour of the drug.

Extraction with solvents.—From 100 g. extracted in a Soxhlet apparatus successively with various solvents the following percentages of extracts dried at 100° were obtained :—Petroleum ether (b.p. 50-60°), 2.0 ; ethyl ether, 1.9 ; chloroform, 1.3 ; ethyl acetate, 15.8 ; acetone, 9.5 and ethyl alcohol (91 per cent.) 12.5 : total, 43.0. Excepting the last two, the extracts consisted of yellow crystalline material. For a detailed examination, the powdered rhizomes (32 kg.) were thoroughly extracted with alcohol (91 per cent.) which was then removed under reduced pressure, the viscous extract being freed from essential oil by steam-distillation. The yellow distillate was extracted several times with ethyl ether, which on subsequent extraction with ammonium and sodium carbonate solutions yielded a very small amount of black material. The sodium hydroxide extract gave a small quantity of chrysophanic acid, m.p. 190-191°.

The ether, after extraction with alkalis, left as residue (25 g.) an orange essential oil which distilled for the most part at 55-60°/10 mm., and a very small portion at 140-145° ; both fractions had in a very

high degree the characteristic smell of the drug. The first fraction was unfortunately lost.

Non-volatile constituents of the extract.

The dark aqueous residue from steam-distillation was decanted and filtered from the brown resinous mass (B), which was washed with water and the washings added to the aqueous filtrate (A), amounting to about 60 litres. Extracts of this with ethyl ether (A1), ethyl acetate (A2) and amyl alcohol (A3), along with the aqueous residue (A4) were examined separately.

Extract (A1) : *Isolation of Rhein*, $C_{15}H_8O_6$.—After removing ether the crystalline residue was recrystallised from amyl alcohol, and then from ethyl alcohol four times, when orange, glistening needles melting at 320-321° were obtained. The acetyl derivative was greenish yellow, melting at 256-257°. Analysis confirmed the above empirical formula for rhein.

Isolation of Emodin, $C_{15}H_{10}O_5$.—The amyl alcoholic filtrate from crude rhein was extracted with 10 per cent. sodium carbonate solution which, on acidification, yielded a reddish brown precipitate (about 15 g.). This, after five crystallisations from ethyl alcohol and finally from pyridine was orange yellow, partially subliming at about 230° and melting to a dark red liquid at 254-255°. The bright yellow acetyl derivative melted at 197-198° and analysis confirmed the formula above (Found : C, 66.8 ; H, 4.1 $C_{15}H_{10}O_5$ requires C, 66.6 ; H, 3.7, per cent.).

The combined mother liquors were concentrated and poured into ethyl ether when some black material settled. The ethereal filtrate was extracted with 10 per cent. ammonium carbonate solution which, on acidification, gave only a dark red viscous product from which no crystalline compound corresponding to rheinolic acid of Tutin and Clewer (*loc. cit.*) could be obtained. After extracting with sodium carbonate, the amyl alcohol solution was extracted with 10 per cent. sodium hyroxide, acidification yielding a product which, when crystallised twice from chloroform, was orange yellow melting at 226-227°. This gave an acetyl derivative which softened at 182° and completely melted at 187°. The amount of the material isolated in a pure form being very small, it could not be further examined.

Isolation of Chrysophanic acid, $C_{15}H_{10}O_4$.—The combined chloroform mother liquors were then extracted first with 0.5 per cent. sodium hydroxide solution (to remove the above compound) and then with 5 per cent. sodium hydroxide ; this on acidification gave a yellow product which after six crystallisations from alcohol formed golden

yellow, shining plates melting at 194-195°. The acetyl derivative melted at 204°, and analysis confirmed the formula of chrysophanic acid.

Extract (A2) : *Isolation of Rhaponticin*, $C_{21}H_{24}O_9$.—The concentrated extract deposited about 200 g. of pale yellow crystals. After being washed once with 20 per cent. ethyl alcohol and recrystallised five times from 5 per cent. alcohol with norit carbon, colourless prismatic crystals melting at 233-234° were obtained. Analysis agreed with the formula $C_{21}H_{24}O_9$, while hydrolysis with dilute sulphuric acid gave glucose and rhapontigenin which, when crystallised thrice from methyl alcohol, yielded colourless glistening needles melting at 184-185°.

Glucoside of chrysophanic acid.—The ethyl acetate filtrate and washings after separating crude rhaponticin were further concentrated to small volume when a brown pulpy mass floated, and was crystallised from 91 per cent. alcohol. The reddish brown product melted at 204-205° and on hydrolysis gave chrysophanic acid (m.p. 192-193°) with a reducing sugar in quantity too small for identification.

Extract (A3) : *Rhaponticin and mixed glucosides.*—After removing the solvent under reduced pressure and digesting the residue with 5 per cent. alcohol, the pale yellow product (about 150 g.) was crystallised several times from 20 per cent. alcohol and became colourless ; melting at 232-233°, it was found to be rhaponticin.

The combined alcoholic filtrates after separating rhaponticin were concentrated to small volume, but the brown amorphous precipitate could not be crystallised. It was therefore hydrolysed with dilute sulphuric acid, giving a reducing sugar and a mixture of anthraquinone derivatives consisting mostly of emodin and chrysophanic acid.

Aqueous residue (A4).—A portion was treated first with neutral lead acetate solution and the filtrate from the heavy precipitate further treated with basic lead acetate solution which gave only a small precipitate. The solids were combined, de-leaded with hydrogen sulphide, and the filtrate from lead sulphide extracted first with ethyl ether and then with amyl alcohol. The ether extract gave a small amount of a brown residue which was strongly acidic to litmus, while the amyl alcoholic extract after removal of solvent under reduced pressure gave a reddish brown residue consisting of mixed glucosides resembling those obtained from A3.

The filtrate from lead precipitate having been de-leaded and decolourised with norit carbon, was concentrated under reduced pressure. The yellow, syrupy residue contained a considerable amount of glucose,

but did not contain any di- or poly-saccharide, the amount of reducing sugar being found unchanged by attempted hydrolysis.

Water-insoluble, brown, resinous mass (B).—The brown viscous mass was well mixed with about 5 kg. of the alcohol-extracted drug, dried completely and extracted in a specially devised continuous extraction apparatus successively with low-boiling petroleum ether (B1), ethyl ether (B2), chloroform (B3) and ethyl acetate (B4). Preliminary work having shown that the acetone and alcoholic extracts gave only a brown, amorphous, tarry material, these extractions were omitted.

Extract (B1).—The golden yellow, crystalline material deposited on concentration was washed with the solvent and crystallised three times from a mixture of chloroform and ethyl acetate (2 :1), when chrysophanic acid (about 20 g.) melting at 193-194° was obtained. The combined filtrates and washings after separating the chrysophanic acid were concentrated, the residue dissolved in ethyl ether and extracted successively with 5 per cent. sodium carbonate and hydroxide solutions to remove emodin and chrysophanic acid, respectively. After evaporating ether the viscous residue was saponified with alcoholic sodium hydroxide solution, the alcohol then distilled, and the residual, strongly alkaline solution, mixed with purified filter-paper pulp, dried and extracted with dry ethyl ether in a Soxhlet apparatus. The unsaponifiable material thus removed, after crystallisation four times from 91 per cent. alcohol, was colourless and melted at 135-136°, giving the common colour reactions of phytosterols and yielding an acetyl derivative melting at 120-121°. From the combined mother liquors, the remaining phytosterol was removed by treating with a hot solution of digitonin, the filtrate from which contained a brown, waxy material.

The Fatty Acids.

After removal of unsaponifiable matter, the soap was extracted from the filter-paper pulp with hot water, and the acids separated as usual into saturated and unsaturated acids by Twitchell's method (*Ind. Eng. Chem.*, 1924, **13**, 806).

Saturated acids.—The methyl esters were prepared in the usual manner and distilled under 2 mm. in three fractions, the acids corresponding to each fraction being crystallised from alcohol. The first (2 g.) on three crystallisations gave a small amount of a product having m.p. 57-58° and M.W. 270, probably a mixture of palmitic and stearic acids. The second (2 g.) and third (0.5 g.) fractions were combined and on being crystallised four times yielded an acid of m.p. 77-78° and M.W. 425. The combustion values (Found : C, 79.6 ; H, 14.1) for this acid however do not agree with those of any of the known acids.

The melting point corresponds to that of cerotic acid while the molecular weight agrees nearly with that of montanic acid. The amount was too small for confirmatory work.

Unsaturated acids.—The methyl esters were distilled under 2 mm. in three fractions from which the yellow colour of some anthraquinone derivatives was removed by washing with cold dilute sodium hydroxide. The first fraction was too small for investigation. The second and third fractions boiled at almost the same temperature, so were combined, hydrolysed and oxidised with cold permanganate solution. The hydroxy-acids thus obtained were extracted in a Soxhlet apparatus successively with petroleum ether, ethyl ether and ethyl alcohol. The petroleum ether yielded only a small amount of a viscous material. The ether extract, on being crystallised twice from ethyl alcohol, gave colourless crystals having m.p. 128-130° and M.W. 318, evidently dihydroxystearic acid. The alcoholic extract on further crystallisation was colourless, having m.p. 165-166° and M.W. 347; this molecular weight agrees well with that of a tetrahydroxystearic acid, but the melting point is lower than that of sativic acid. A lower melting point for tetrahydroxystearic acid has been observed before by several investigators, and it is therefore possible that this product is a mixture of isomeric acids having the formula, $C_{18}H_{36}O_6$.

THE EXTRACTS, B_2, B_3 *and* B_4.

Extract (B2).—Concentration gave a dark red solution with a large proportion of yellow solid which was thoroughly washed with ether and crystallised six times from hot alcohol; the lustrous, golden yellow plates melting at 194-195° consisted of chrysophanic acid. The residue from the combined alcoholic mother liquors was crystallised several times from pyridine; the deep orange yellow needles melting at 254-255° consisted of emodin.

Extract (B3).—The brown viscous residue after removing the solvent was washed with cold alcohol several times, the washings on concentration depositing a small amount of emodin. The alcohol-insoluble residue was digested with cold sodium carbonate solution and filtered, acidification giving a yellow, crystalline material which when recrystallised several times from pyridine yielded rhein melting at 320-321°. The sodium carbonate-insoluble material was well washed with acidulated water, crystallised many times from a mixture of ethyl acetate and chloroform (1 :1) and finally from pyridine when golden yellow chrysophanic acid melting at 193-194° was obtained.

Extract (B4).—Concentration gave yellow crystals with brownish granules. After repeated washing with ethyl alcohol the granules were

separated partly by hand-picking and partly by flotation. The yellow material on several crystallisations from dilute alcohol with norit carbon, became colourless, and was identified as rhaponticin (m.p. 230-231°). The brown granules when crystallised three times from alcohol melted at 207-208°, and on hydrolysis with dilute sulphuric acid gave a reducing sugar with impure chrysophanic acid. The alcoholic washings of the solid material described above gave emodin, m.p. 253°.

SUMMARY.

The alcoholic extract of the rhizomes of *Rheum emodi,* Wall. has been found to contain the following :—Starch, tannin material, glucose, a small amount of volatile organic acids, an essential oil, chrysophanic acid, rhein, emodin, another hydroxyanthraquinone (probably aloe-emodin), the glucoside rhaponticin, and a mixture of glucosides of hydroxyanthraquinones from which chrysophanic acid glucoside has been isolated in a fairly pure form ; also oleic and linoleic acids, probably a mixture of palmitic and stearic acids along with a small amount of an unidentified acid of high molecular weight, phytosterol, brown resinous matter, and a large amount of brown amorphous powder resembling plant pigments.

Of the anthraquinone derivatives, chrysophanic acid was found to occur in the largest amount followed by emodin, rhein and the unidentified hydroxyanthraquinone derivative. The glucosides of hydroxyanthraquinones were also present in fairly large quantities. The minimum yield of rhaponticin was found to be about 0.7 per cent.

The results show that the Indian or Himalayan rhubarb contains most of the anthraquinone derivatives found so far in the official rhubarb (*Rheum palmatum,* Linn. or *Rheum officinale,* Baill.), together with an appreciable amount of the glucoside rhaponticin, which is absent from all the official rhubarb samples so far analysed. The Central Indigenous Drugs Committee (*loc. cit.*) found the drug unsatisfactory as a purgative but adduced no reasons for this. It would appear from the results of the present investigation that the griping action of the preparations from this drug is probably due to the presence of rhaponticin which is present to the extent of nearly one per cent. In order to throw more light on this aspect of the subject, rhaponticin is being subjected to a detailed pharmacological study, the results of which will be communicated in due course.

We desire to record our appreciation of the keen interest shown by Professor V. Subrahmanyan in the present investigation ; one of

us (M.G.M.) wishes also to express his grateful thanks to the Osmania University Council and the Principal of the Osmania Medical College, Hyderabad (Deccan) for having deputed him to work in these laboratories.

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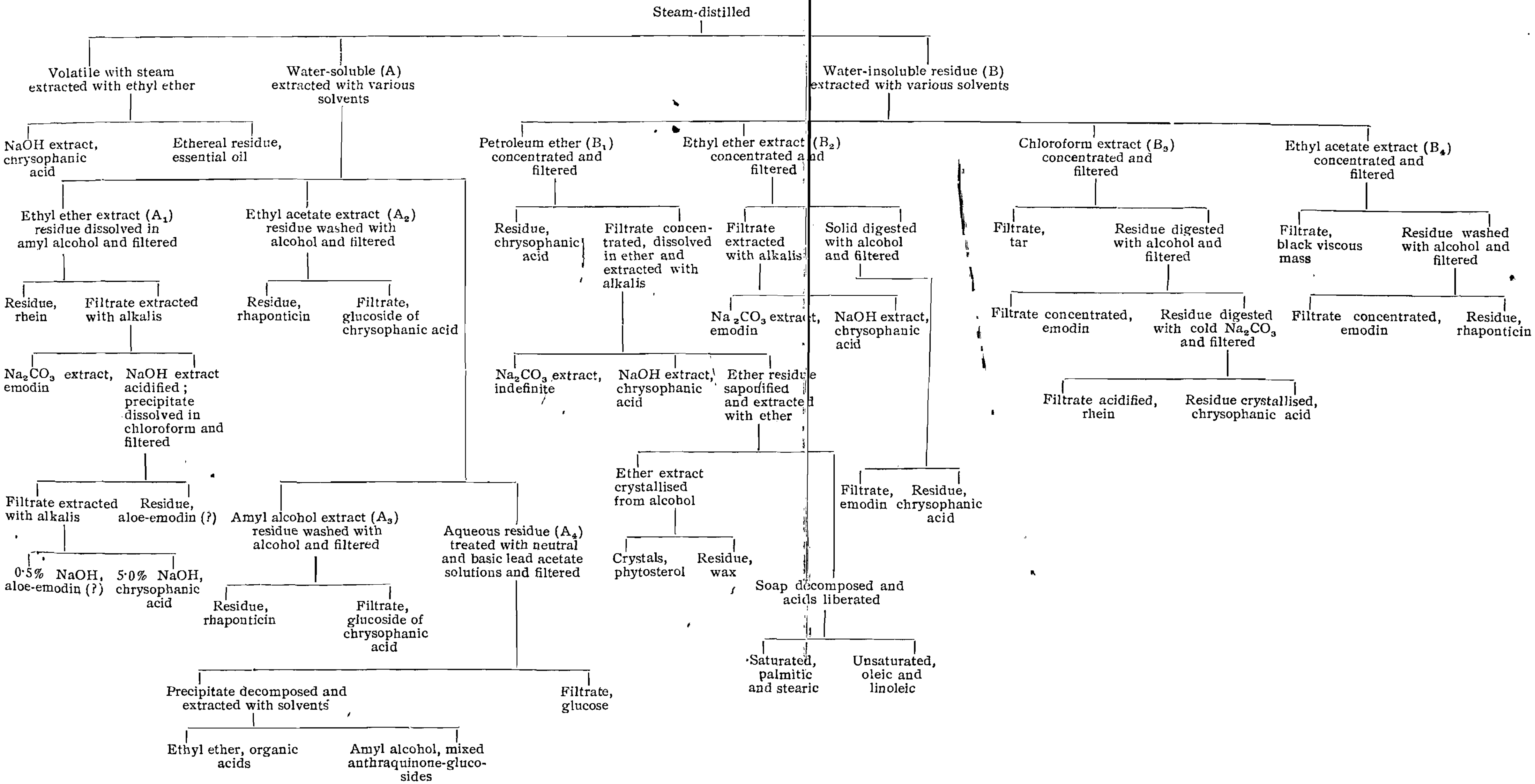
DIAGRAMMATIC ANALYSIS OF ALCOHOLIC EXTRACT FROM INDIAN OR HIMALAYAN RHUBARB (*RHEUM EMODI*, WALL.)



CONSTITUTION OF THE SO-CALLED DITHIOURAZOLE OF MARTIN FREUND.

PART V. ISOMERISM OF HYDRAZODITHIODICARBONAMIDES, IMINOTHIOLTHIOBIAZOLES AND DI-R-IMINOTHIOBIAZOLES.

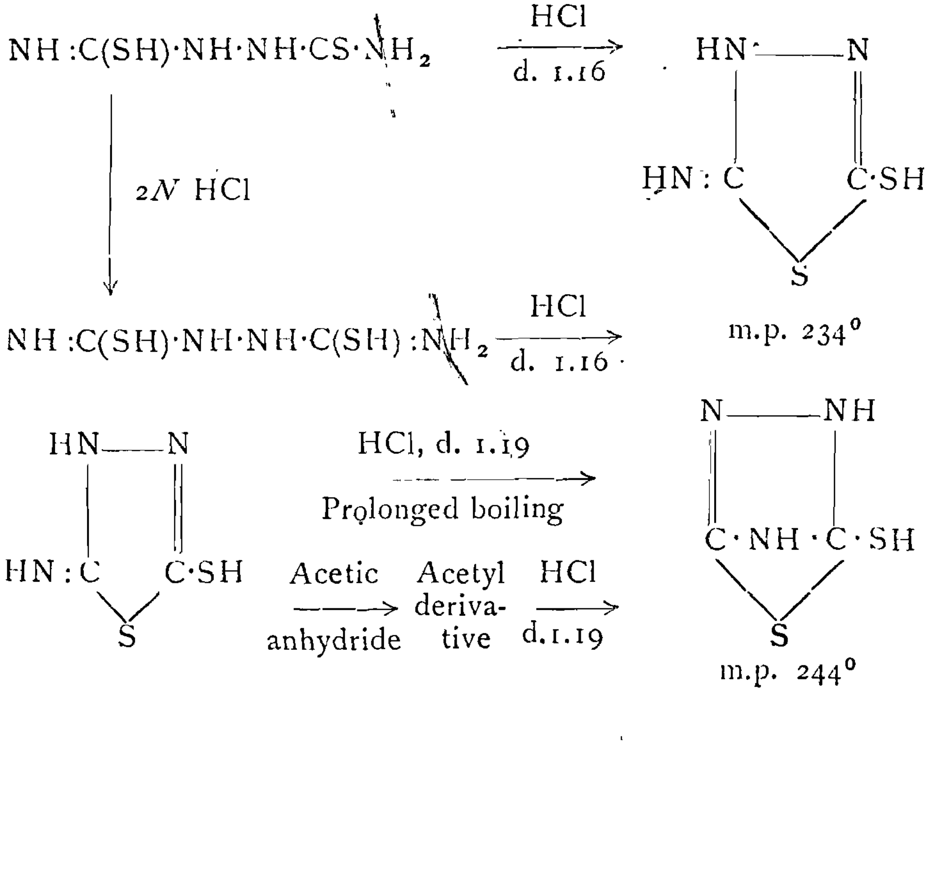
By Shaha L. Janniah and P. C. Guha.

In part IV (*J. Amer. Chem. Soc.*, 1930, **52**, 4860) hydrazodithiodicarbonamide has been found to exist in two tautomeric forms,

$$NH_2\text{-}CS\text{-}NH\text{-}NH\text{-}C(SH):NH$$

$$\text{and } NH:C(SH)\text{-}NH\text{-}NH\text{-}C(SH):NH,$$

and their interconvertibility established. Iminothiolthiobiazole has been isolated as an intermediate compound during the formation of Freund's so-called dithiourazole (*Ber.*, 1895, **28**, 946) which has been shown to be *endo*iminothiolthiobiazole; the latter has been found to be formed either by the prolonged action of hydrochloric acid (d. 1.19) on either the hydrazide or the iminothiolthiobiazole, or by acetic anhydride treatment of the thiobiazole with subsequent deacetylation.



In the present paper, the action of hydrochloric acid of different strengths and of acetic anhydride has been studied upon the mono- and di-substituted hydrazodithiodicarbonamide of the types,

$NHR-CS-NH-NH-CS-NH_2$ and $NHR-CS-NH-NH-CS-NHR$ and the isomeric thiobiazoles obtained from them by the action of hydrochloric acid of different strengths, and of acetic anhydride have been studied.

The action of 2*N* hydrochloric acid upon the mono- and di-substituted hydrazides is the same as with the unsubstituted hydrazide (described in part IV) and thus *sym*-dimethyl, mono-phenyl, *sym*-diphenyl and *sym*-di-*o*-tolylhydrazodithiodicarbonamides have yielded isomeric compounds I-IV.

NMe : C(SH)-NH-NH-C (SH) : NMe
(I)

NPh : C(SH)-NH-NH-C (SH) : NH
(II)

NPh : C(SH)-NH-NH-C (SH) : NH
(III)

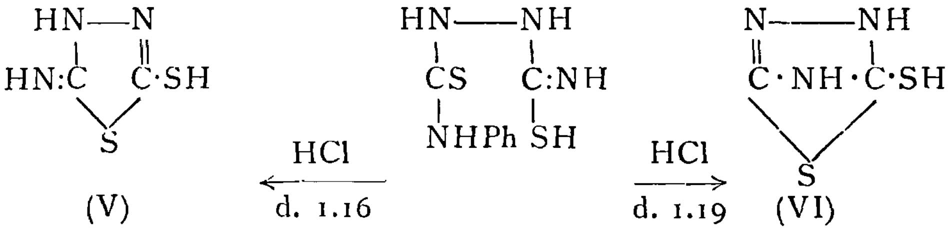
$CH_3.C_6H_4N$: C(SH)-NH-NH-C (SH) : $NC_6H_4 \cdot CH_3$
(IV)

The isomeric dimethylhydrazides give quite different absorption bands thus fulfilling the expectation that substituted hydrazides resemble the unsubstituted in undergoing isomeric change. The absorption spectra of the isomeric aryl-substituted hydrazides have not been studied as their aryl groups were likely to produce complicated absorption bands in addition to those due to the labile hydrogen atoms in the $CS.NH_2$ group of the hydrazides.

Attempts to obtain heterocyclic compounds of the types III and IV mentioned in part I from the mono-substituted hydrazides were unsuccessful; both hydrochloric acid and acetic anhydride eliminate the corresponding amines forming unsubstituted thiobiazoles. Monophenylhydrazodithiodicarbonamide (m.p. 180°) and its isomer (m.p. 162°) give iminothiolthiobiazole, m.p. 234° (V) and the *endo*iminothiolthiobiazole, m.p. 245° (VI) with hydrochloric acid of d. 1.16 and 1.19 respectively, conditions of experiments scarcely differing from those of their formation from the unsubstituted hydrazide,

NH_2-CS-NH-NH-CS-NH_2

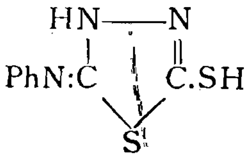
The reaction evidently proceeds in the following manner :



(V) (VI)

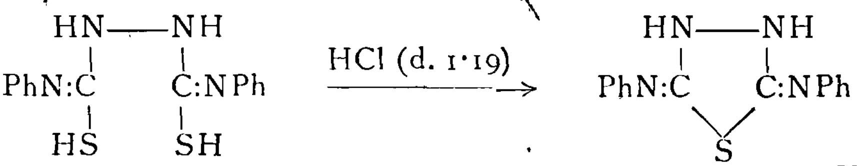
The literature does not appear to describe the action of acids upon the mono-substituted hydrazodithiodicarbonamides.

The symmetrical disubstituted hydrazides have given interesting results. Freund and Imgart (*Ber.*, 1895, **28**, 946) obtained by the action of hydrochloric acid upon diphenylhydrazodithiodicarbonamide an alkali-soluble compound, $C_8H_7N_3S_2$, melting at 210° which they called phenyldithiourazole, proved subsequently by Guha to be 3-thiol-5-phenylimino-4 :1 :2-thiobiazole (VII).



(VII)

It has now been found, however, that this thiobiazole is formed in good yield only when the hydrazide is treated with hydrochloric acid (d. 1.16) and not with concentrated acid (d. 1.19), in which case an alkali-insoluble compound, $C_{14}H_{12}N_4S$, m.p.249° is obtained as the main product. This contains two phenyl groups, and the sulphur atom is a member of the ring. It is therefore formed by the elimination of a molecule of hydrogen sulphide from the diphenylhydrazide thus :

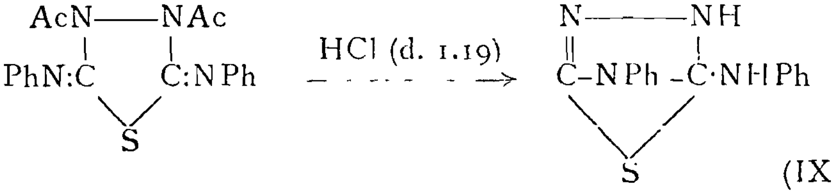


(VIII)

Identity of this (VIII) with that obtained by Guha from diphenylhydrazodithiodicarbonamide and acetic anhydride (*J. Amer. Chem. Soc.*, 1923, **45**, 1036) has been established.

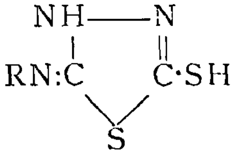
It is, however, interesting to note that the acetyl derivative (m.p. 224°) of 3 :5-diphenyliminthiobiazole, whether obtained from the diphenylhydrazide or the thiobiazole by the action of acetic anhydride, regenerates the same thiobiazole melting at 249° when treated with dilute hydrochloric acid (1 :1). If concentrated hydrochloric acid (d. 1.19) is used as hydrolysing agent, an alkali-insoluble thiobiazole (IX) melting at 208° and isomeric with (VIII) and with the thiobiazole, m.p.249°, is obtained. This product has escaped previous workers because they used less concentrated hydrochloric acid. Since Guha employed hydrochloric acid of which the strength is not mentioned, it is probable that his sample was mixed with a trace of the 208° variety, thus accounting for the slightly lower melting point (viz., 247°) recorded for the 249° variety.

Compounds (VIII) and (IX) contain two phenyl groups and one sulphur atom ; they are insoluble in alkali, do not form disulphides and are not desulphurised by mercuric oxide. Guha (*J. Amer. Chem. Soc.*, 1923, **45**, 1040) has already proved the constitution of the thiobiazole (VIII) and shown it to be 3 :5-diphenylimino-4 :1 :2-thiobiazole. By analogy with the compounds discussed in part IV, the new thiobiazole melting at 208° is in all probability, 2 :3-dihydro-3-anilino-3 :5-*endo*phenylimino-4 :1 :2-thiobiazole, formed from the acetyl derivative of compound (VIII) by molecular rearrangement during hydrolysis with acid (d. 1.19) :—

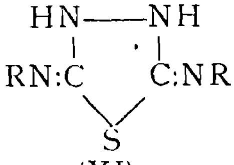


(IX)

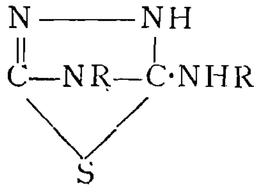
Di-*o*-tolylhydrazodithiodicarbonamide resembles its phenyl analogue and yields three thiobiazole compounds X, XI and XII :—



(X)



(XI)



(XII) (R = *o*-tolyl)

EXPERIMENTAL.

Isomer of dimethylhydrazodithiodicarbonamide (I).—Dimethylhydrazodithiodicarbonamide (5 g.), prepared according to the method of Freund (*Ber.*, 1895, **28**, 946), was dissolved in 2*N* hydrochloric acid (100 c.c.) over a small flame and the solution boiled for exactly ten minutes. On slow cooling minute needles separated and were recrystallised from hot water in colourless needles, m.p. 198°, the yield being almost quantitative. This substance was found to be more readily soluble in alkali than the parent hydrazide (m.p.224°) and the analytical values were the same (Found : N, 31.6 ; S, 36.0 ; $C_4H_{10}N_4S_2$ requires N, 31.4 ; S, 35.9 per cent.). The absorption spectra of both have been studied in aqueous solutions.

Isomer of monophenylhydrazodithiodicarbonamide (II).—Experimental procedure was that adopted in the case of the unsubstituted hydrazide (*J. Amer. Chem. Soc.*, 1930, **52**, 4864). The crystalline solid obtained on cooling the solution from 3 g. of the hydrazomonophenyldithiodicarbonamide (m.p. 180°) in 2*N* acid (80 c.c.) was filtered, washed with water and recrystallised from dilute alcohol, m.p. 162° ; yield, 1.6 g. (Found : N, 24.9 ; S, 28.5. $C_8H_{10}N_4S_2$ requires N, 24.7 ; S, 28.31 per cent.).

Action of strong hydrochloric acid on monophenylhydrazodithiodicarbonamide : Formation of compounds V *and* VI.—No phenylthiobiazole could be obtained from the hydrazide by treating it with hydrochloric acid of various higher concentrations in the manner described in part IV ; aniline was liberated in all cases together with a small quantity of phenyl mustard oil. Only iminothiolthiobiazole (m.p. 234°) was obtained as the final product with hydrochloric acid (d. 1.16) and *endo*iminothiolthiobiazole (m.p. 244°) with acetic anhydride followed by deacetylation with hydrochloric acid (d. 1.19) or direct treatment with the latter (Found : N, 31.5 ; S, 47.9. $C_2H_3N_3S_2$ requires N, 31.6 ; S, 48.1 per cent.).

Isomer of diphenylhydrazodithiodicarbonamide (III).—Diphenylhydrazodithiodicarbonamide (m.p. 187°) on treatment with 2*N* hydrochloric acid gave an isomeric hydrazide, which on recrystallisation from hot dilute alcohol melted sharply at 173°. The mixed melting point of the two hydrazides was quite indefinite, gradually rising from 155° to about 180° (Found : N, 18.6 ; S, 21.23. $C_{14}H_{14}N_4S_2$ requires N, 18.54 ; S, 21.19 per cent.)

Diphenylhydrazodithiodicarbonamide and hydrochloric acid (d. 1.16) ; *Formation of* 3-*thiol*-1 :5-*dihydro*-5-*phenylimino*-4 :1 :2-*thiobiazole* (VII).—The hydrazide (3 g.) soon dissolved in the boiling acid

(20 c.c.), but after 3-4 minutes some reddish-brown, oily drops separated with odour of phenyl mustard oil; after about ten minutes the solution became turbid and a white solid separated rapidly on cooling. This was filtered, well pressed, washed with a little water and ether to remove mustard oil, and finally recrystallised from 95 per cent. alcohol, m.p.219°. Yield, 1.8 g. It dissolved easily in cold dilute alkali (Found: N, 20.4; S, 30.2. $C_8H_7N_3S_2$ requires N, 20.1; S, 30.6 per cent.).

3:5-*Diphenylimino*-2:3:5:1-*tetrahydro*-4:1:2-*thiobiazole* (VIII). Diphenylhydrazodithiodicarbonamide (3 g.) dissolved when gently heated with concentrated acid (d. 1.19, 20 c.c.); after boiling over a small flame for nearly fifteen minutes a very small quantity of phenyl *iso*thiocyanate was liberated. The clear, filtered solution when cold gave a curdy white solid which after being washed with water and ether was crystallised from 95 per cent. alcohol, m.p. 249° (decomp.). Yield, 1.2 g. The substance was insoluble in cold dilute alkali but was decomposed when boiled with sodium hydroxide solution liberating phenyl *iso*cyanide. It did not give any disulphide with iodine, which was not even decolorised, and could not be desulphurised by mercuric oxide (Found: N, 21.2; S, 11.6. $C_{14}H_{12}N_4S$ requires N, 20.9; S, 11.9 per cent.). The strongly acid filtrate on being neutralised with dilute ammonia gave a small quantity of the alkali-soluble phenyliminothiolthiobiazole melting at 219°.

The *diacetyl* derivative, prepared by heating the diphenyliminothiodiazole, m.p. 249°, with acetic anhydride, crystallised from hot water acidified with acetic acid, m.p. 224°. It is identical with that obtained by treating the diphenylhydrazodithiodicarbonamide (m.p. 187°) with acetic anhydride (Found: N, 16.2; S, 8.9. $C_{18}H_{16}O_2N_4S$ requires N, 15.9; S, 9.09 per cent.).

Hydrolysis of the diacetyl derivative with 5N *hydrochloric acid*: *Formation of compound* (VIII).—The diacetyl derivative dissolved after about five minutes in the boiling acid the solution becoming turbid during the next ten minutes. After crystallisation from alcohol the product was found to be the diphenyliminothiobiazole, m.p. 249°.

The endo*phenyliminothiobiazole* (IX).—When the diacetyl derivative was hydrolysed by concentrated hydrochloric acid (d. 1.19) the solution did not become turbid as with 5*N* acid, although the boiling was prolonged for about twenty minutes. Drops of phenyl mustard oil separated, and the solution on cooling deposited white crystals which separated from absolute alcohol in cubes, m.p. 208°. It was insoluble in sodium hydroxide solution, but decomposed on boiling with the alkali and could not be desulphurised by mercuric oxide (Found: N, 21.15; S, 11.7. $C_{14}H_{12}N_4S$ requires N, 20.9; S, 11.9 per cent.).

*Isomer of di-*o*-tolylhydrazodithiodicarbonamide* (IV).—On treatment with 2*N* hydrochloric acid, the ordinary hydrazide (m.p. 171°) yielded the isomeric compound, obtained from dilute alcohol as minute, colourless crystals m.p. 178°. The melting point of a mixture with the original hydrazide ranged from about 162° to 176° (Found: N, 16.8; S, 19.2. $C_{16}H_{18}N_4S_2$ requires N, 16.99; S, 19.4 per cent.).

o*-Ditolylhydrazodithiodicarbonamide and strong hydrochloric acid* (d. 1.16): *Formation of* 3*-thiol-*5 :1*-dihydro-*5*-tolylimino-*4 :1 :2*-thiobiazole* (X).—The hydrazide soon dissolved in the hot acid and further boiling of the solution gave a white solid with a few drops of tolyl mustard oil. At this stage the mixture was allowed to cool and the solid washed with water and then with a little ether; it crystallised from 95 per cent. alcohol in minute, colourless prisms m.p. 195°. This thiobiazole was readily soluble in cold dilute alkali and decolourised iodine with the formation of a disulphide (Found: N, 19.1; S, 28.4. $C_9H_9N_3S_2$ requires N, 18.8; S, 28.7 per cent.).

3 :5*-Di-*o*-tolylimino-*2 :3 :5 :1*-tetrahydro-*4 :1 :2*-thiobiazole* (XI). —On heating, di-*o*-tolyl-hydrazodithiodicarbonamide soon dissolved in boiling concentrated hydrochloric acid (d. 1.19) and a very small quantity of the *iso*thiocyanate was liberated. The clear solution gave on cooling a white solid, which after being washed with water and ether was crystallised from 95 per cent. alcohol, m.p. 217° (decomp.) (Found: N, 19.2; S, 11.1. $C_{16}H_{16}N_4S$ requires N, 18.9; S, 10.8 per cent.). The substance was insoluble in cold dilute alkali, but decomposed on boiling and did not give a disulphide. As in the case of the diphenylhydrazide the acid filtrate from the foregoing substance on neutralisation gave a white solid which crystallised from alcohol, m.p. 195° and was identical with the alkali-soluble thiobiazole (X).

The *diacetyl* derivative from the above thiobiazole (m.p. 217°) after crystallisation from dilute acetic acid melted with decomposition at 262° (Found; N, 14.4; S, 8.2. $C_{20}H_{20}O_2N_4S$ requires N, 14.7; S, 8.4 per cent.).

Hydrolysis of the diacetyl derivative with 5N *hydrochloric acid*: *Formation of* 3 :5*-ditolyliminothiobiazole* (XI).—The boiling acid dissolved the diacetyl derivative in about five minutes, and after another ten minutes the clear solution became turbid; when cold, the solid product was filtered, washed, and recrystallised from alcohol, m.p. 217°, being identical with the di-*o*-tolyliminothiobiazole.

Hydrolysis with concentrated acid (d. 1.19): *Formation of* 2 :3*-dihydro-*3*-tolylimino-*3 :5-endo-o*-tolylimino-*4 :1 :2*-thiobiazole* (XII).—The clear solution obtained on boiling the diacetyl derivative with the

acid deposited white cubic crystals on cooling. These after recrystallisation from absolute alcohol melted at 133°. It was insoluble in cold dilute alkali but decomposed on boiling and did not decolourise iodine (Found: N, 18.8; S, 11.0. $C_{16}H_{16}N_4S$ requires N, 18.9; S, 10.8 per cent.).

It is to be mentioned that both of the isomeric monophenyl, diphenyl- and di-*o*-tolylhydrazodithiodicarbonamides have been found to yield identical compounds on treatment with hydrochloric acid of density 1.16 and 1.19, and acetic anhydride respectively.

SUMMARY

The action of 2*N* hydrochloric acid on mono- and di-substituted hydrazodithiodicarbonamides is the same as on the unsubstituted hydrazide in giving the corresponding isomers. Stronger acids eliminate the amine from the mono-substituted hydrazide yielding the isomeric iminothiol- and *endo*iminothiolthiobiazoles (V) and (VI).

In the case of disubstituted hydrazides, hydrochloric acid (d. 1.16) gives the alkali-soluble R-iminothiolthiobiazole (VII) as the main product; concentrated acid (d. 1.19) on the other hand yields isomeric 3:5-di-R-iminothiobiazole (VIII) and 3:5-*endo*-R-imino-3-R-iminothiobiazole (IX).

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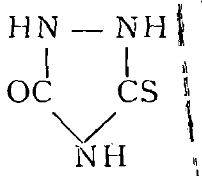
[Accepted, 18-1-33.]

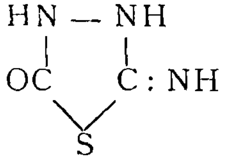
PART VI. ISOMERISM OF HYDRAZOMONOTHIODICARBONAMIDES, IMINOTHIOBIAZOLONES AND MONOTHIOURAZOLES.

By S. L. Janniah and P. C. Guha.

Freund and Schander (*Ber.*, 1896, **29**, 2506), by heating the hydrazide NH_2-CS-NH-NH-CO-NH_2 with concentrated hydrochloric acid obtained a compound, $C_2H_3ON_3S$, to which they ascribed the monothiourazole formula. Busch and Lotz (*J. pr. Chem.*, 1914, **90**, ii, 257) have shown Freund's monothiourazole to be 3-imino-4 :1 :2-thiobiazole-5-one



(Freund)



(Busch and Lotz)

Arndt, Milde, and Tschenscher (*Ber.*, 1922, **55**, 341) found that of the two isomeric hydrazides,

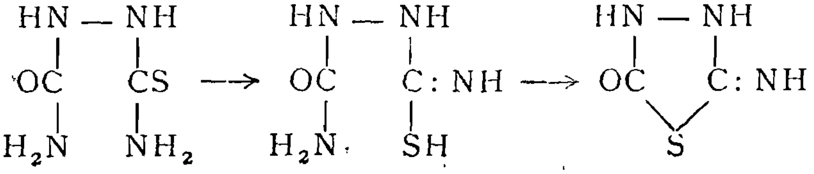
NHPh-CO-NH-NH-CS-NH_2 and NHPh-CS-NH-NH-CO-NH_2

the former gave thiourazole (m.p. 206°) and 4-phenylthiourazole, and the latter phenylthiourazole, on being boiled with a solution of sodium hydroxide.

While attempting the ring-closure of hydrazomonothio- (and dithio-) dicarbonamides with acetic anhydride, Guha (*J. Amer. Chem. Soc.*, 1923, **45**, 1036) found that the hydrazide

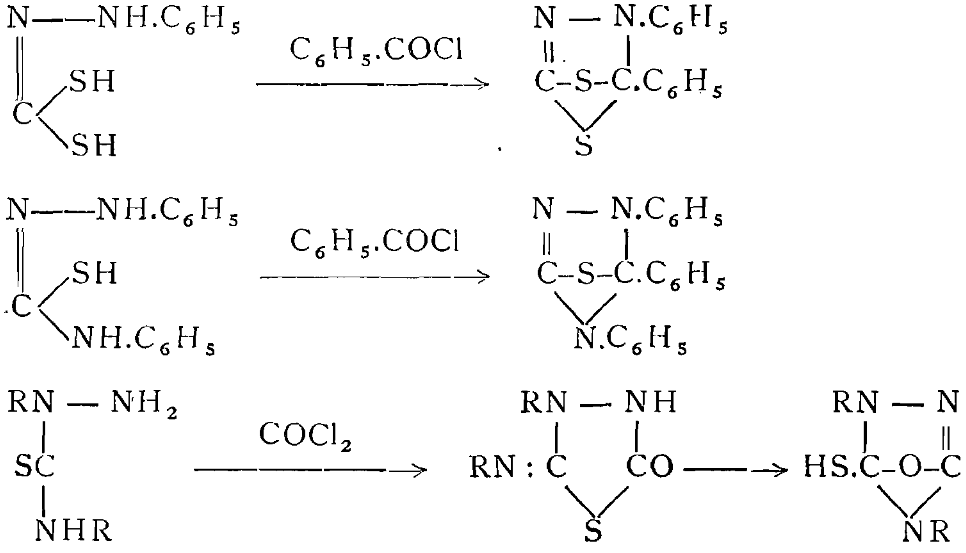
NH_2-CS-NH-NH-CO-NH_2

instead of giving 3 :5-diiminotetrahydro-4 :1 :2-thiobiazole according to the general rule holding good in the case of the hydrazodithiodicarbonamides, gave ketoiminothiodiazole m.p. 235°, thus :



and that his compound was quite different from Freund's compound (m.p. 177°).

Guha and Sen (*J. Ind. Chem. Soc.*, 1927, **4,** 43) obtained by the action of urea upon substituted thiosemicarbazides four types of compounds namely, 3-R-5-keto-3-thioldihydro-4 :1 :2-triazole, 5-keto-3-R-aminodihydro-4 :1 2-thiobiazole, 4-R-3-thiol-5-R-amino-4 :1 :2-triazole and 3 :5-*endo*xy-4 :1 :2-triazole. Of the above four types, ketothioltriazoles have been prepared also by Freund and Schneider, Arndt and his collaborators (*loc. cit.*) and by Fromm and Nehring *Ber.*, 1923, **56**, 1370) ketoaminothiobiazoles by Busch (*Ber.*, 1901, **34**, 320, 2328 ; 1902, **35**, 973 ; 1904, **37**, 2333 ; 1909, **42**, 4763 ; 1911, **44,** 561, 1580) and by Nirdlinger and Acree (*J. Amer. Chem. Soc.*, 1922, **44,** 224). Lastly, though Schneider (*J. pr. Chem.*, 1890, **67,** 263), Marckwald (*Ber.*, 1892, **25,** 3113 ; 1896, **29,** 2923), Busch (*loc. cit.*) and Nirdlinger and Acree have prepared the derivatives of *endo*xy-, *endo*thio-, and *endo*iminotriazoles and thiobiazoles by methods fundamentally different from that of Guha, e.g :—

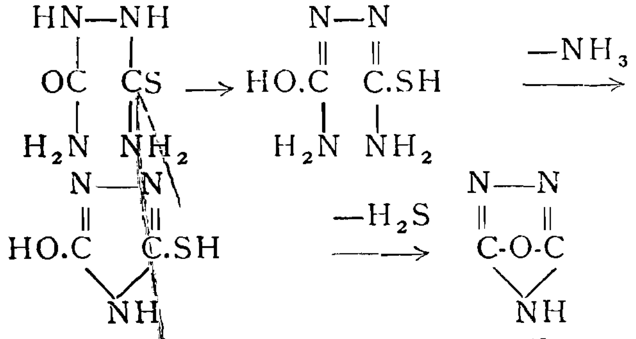


the parent *endo*xytriazole was prepared for the first time by Guha and Sen. Guha and Chakraborti (*J. Ind. Chem. Soc.*, **6**, 99-110) while studying the ring-closure of hydrazomonothiodicarbonamide and its mono- and *sym*-di-substitution products with acetic anhydride, obtained iminothiobiazolones and iminothioltriazoles.

The present investigation is a continuation of the work described in parts IV and V, undertaken with the expectation that interesting cases of isomerism amongst the hydrazomonothiodicarbonamides, as also

amongst the thiobiazoles and triazoles obtained from them by ring-closure might yet arise, and these have been found.

Our first attempt was to study the effect of 2*N* hydrochloric acid upon hydrazomonothiodicarbonamide, but this failed to give an isomer ; under the various conditions tried only thiosemicarbazide hydrochloride (m.p. 190°) could be obtained. With boiling 5*N* hydrochloric acid, however, the hydrazide gave a substance, C_2HON_3, m.p. 255°, identical with 3 :5-*endo*xy-4 :1 :2-triazole prepared by Guha and Sen (*J. Ind. Chem. Soc.*, 1927, **4**, 43). The reaction evidently takes the following course :—

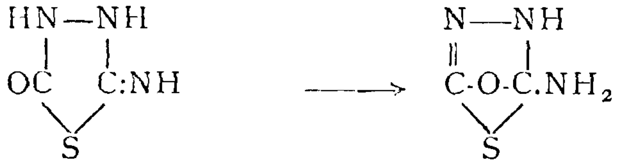


(I)

By repeating Freund's experiment with hydrochloric acid (d. 1.16) under varied experimental conditions it has now been found that iminothiobiazolone, m.p. 177°, is formed in poor yield ; and with concentrated hydrochloric acid (d. 1.19) both compounds (m.p. 177° and 235°) are produced, the latter due to its pronounced basic property remaining in the acidic mother liquor after the separation of the former.

This thiobiazolone (m.p. 177°) gives with acetic anhydride an acetyl derivative (m.p. 295°) which on being hydrolysed with concentrated hydrochloric acid gives a hydrochloride (m.p. 107-108°), and a free base (m.p. 235°) identical with the corresponding compounds obtained from hydrazomonothiodicarbonamide with acetic anhydride (Guha, *J. Amer. Chem. Soc.*, 1923, **45**, 1036). The substance has the composition, $C_2H_3ON_3S$, and is therefore isomeric with the substance melting at 177°. It is insoluble in alkali, remains unchanged on mercuric oxide treatment, and forms compounds with phenyl *iso*-cyanate, phenyl mustard oil, and benzaldehyde. It is thus clear that (1) the sulphur atom is a member of the ring, and (2) an amino-group is present. All these facts give further support to the *endo*aminothiobiazole structure (III) ascribed to compound, m.p. 235° (compare part IV, 4863). It appears very probable that by the action of concen-

trated hydrochloric acid the hydrazide is first converted into iminothiobiazolone, m.p. 177° (II) which then rearranges itself into the *endo*-oxyaminothiobiazole, m.p. 235° (III) :—



(II) (III)

Mono-substituted hydrazides of the type,

NH_2-CO-NH-NH-CS-NHR (R = Ph, Me, Allyl).

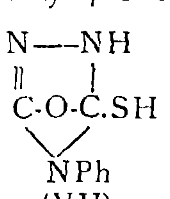
give the corresponding isomeric hydrazides (IV, V, VI) with 2*N* hydrochloric acid similar to those described in parts IV and V.

NHPh-CS-NH-NH-CO-NH_2 (IV)

CH_3·NH-CS-NH-NH-CO-NH_2 (V)

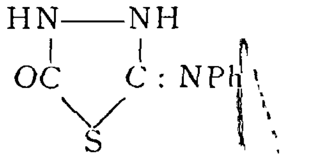
C_3H_5·NH-CS-NH-NH-CO-NH_2 (VI)

Although hydrochloric acid of different strength (d. 1.16 and 1.19) produces different thiobiazoles in the case of the mono- and di-substituted hydrazodithiodicarbonamides, their effect as ring-closing agents on the hydrazides of the monothio-series is similar. Strong hydrochloric acid brings about a ring-closure in the hydrazide (IV) producing a compound (m.p. 184°) of the empirical formula, $C_8H_7ON_3S$. This is soluble in dilute alkali, gives a thiomethyl ether, m.p. 90-91°, and a disulphide, m.p. 196°. These properties eliminate the possibility of the compound being 3-phenylimino-5-keto-4 :1 :2-thiobiazole (m.p. 246°) synthesised by Guha and Sen (*J. Ind. Chem. Soc.*, 1927, **4**, 44) by the action of urea on phenylthiosemicarbazide. It therefore follows that the substance under consideration must be a triazole containing a thiol group. 4-phenylthiourazole (m.p. 196°) of Arndt (*Ber.*, 1922, **55**, 341), obtained by the action of alkali on the same hydrazide has a mercaptanic group (thiomethyl ether m.p. 208°, disulphide, m.p. 285°), and is evidently a different substance. There thus remains the only alternative *endo*-formula (VII) for the substance melting at 184°, viz., 3-thiol-3 :5-*endo*xy-2 :3-dihydro-4-phenyl-4 :1 :2-triazole,

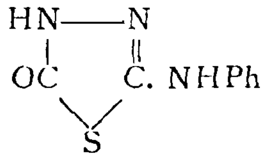


(VII)

Compound (VII) gives with acetic anhydride a diacetyl derivative (m.p. 213-214°) which on deacetylation gives a substance (m.p. 206°) having the same composition ($C_8H_7ON_3S$) as the parent *endo*xytriazolethiol but without mercaptanic properties. Thus compound (VII) after acetylation and deacetylation is no longer a triazole, and must have been changed into a substance with the sulphur atom in the ring. The diacetyl derivative (m.p. 214°) and the deacetylated compound (m.p. 206°) have been proved identical with the corresponding compounds obtained by Guha and Chakraborti from the hydrazide by the action of acetic anhydride (*loc. cit.*). To distinguish their compound, m.p. 206°, from that having m.p. 246° (Guha and Sen, *loc. cit.*) Guha and Chakraborti ascribed a ketophenyliminotetrahydrothiobiazole structure to the former as distinct from the ketophenylaminodihydrothiobiazole structure of the latter

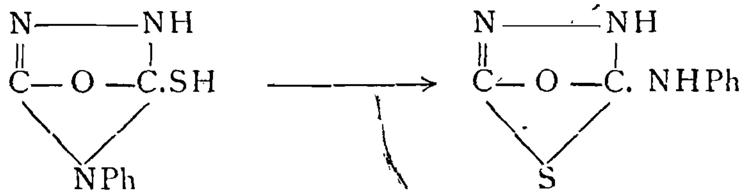


(m.p. 206°, Guha and Chakraborti)



(m.p. 246°, Guha and Sen)

Seeing that both compounds (m.p. 206° and 246°) are quite stable and show no sign of interconvertibility under ordinary conditions, unexpectedly from the tautomeric nature of the above formulae, and from analogy with the *endo*-isomers of the thiobiazoles and triazoles obtained from the hydrazomono- and dithiodicarbonamides, it is now proposed to attribute an *endo*-formula, namely, 3-phenylamino-3 :5-*endo*xy-4 :1 :2-thiobiazole, to the compound melting at 206° ; and its formation from compound (VII) is explained on the assumption that an interchange of position between NPh and S takes place :—



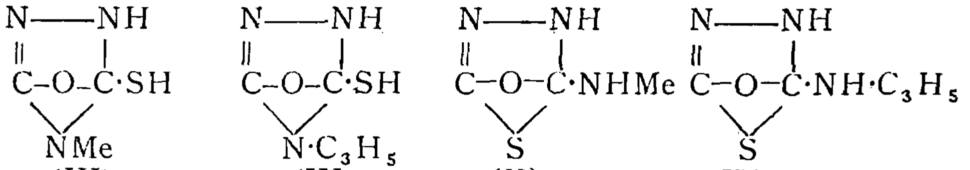
(VII) (VIII)

Methyl- and allyl-substituted hydrazomonothiodicarbonamides,

NH_2-CO-NH-NH-CS-NHR (R = Me, Allyl),

by the action of concentrated hydrochloric acid and acetic anyhdride, have yielded the corresponding methyl and allyl substituted *endo*xy-

thioltriazoles (IX and XI) and *endoxy*-R-iminothiobiazoles (X and XII) respectively :



(IX) (XI) (X) (XII)

EXPERIMENTAL.

Hydrazomonothiodicarbonamide and 5N *hydrochloric acid* : *Formation of* 3 :5-end*oxy*-4 :1 :2-*triazole* (I).—The hydrazide (m.p. 220° ; 5 g.) was heated with the acid (50 c.c.) and the clear solution thus obtained was boiled during half an hour, when hydrogen sulphide and a small quantity of sulphur separated. The clear solution on cooling gave a small quantity of white crystalline solid, increased on concentrating the mother liquor. This crystallised from hot water, m.p. 255° ; yield, about 2 g. The substance was insoluble in alkali and was identical with the *endo*xytriazole (m.p. 255°) of Guha and Sen (*loc. cit.*) (Found : N, 50.85 ; C_2HON_3 requires N, 50.6 per cent.).

Hydrazomonothiodicarbonamide and concentrated hydrochloric acid (d. 1.19) : *Formation of* 3-*imino*-5-*keto*-4 :1 :2-*thiobiazole*.—The hydrazide (5 g.) was boiled vigorously with the acid (25 c.c.) during half an hour when sulphuretted hydrogen was evolved profusely. On cooling, a very small quantity of a crystalline solid separated which crystallised from hot water, m.p. 255°, and was identical with the *endo*xytriazole obtained above. The mother liquor on concentration gave a little more of the *endo*xytriazole and the filtrate from the latter on being evaporated to dryness left a white residue which crystallised from a small quantity of water, m.p. 177°. The yield was only about 0.5 g., but the hydrazide, when gently boiled with hydrochloric acid (d. 1.19) for only 15 minutes, gives a better yield of 177° compound (Found : N, 36.3 ; S, 27.5. $C_2H_3ON_3S$ requires N, 35.9 ; S, 27.35 per cent.).

Iminothiobiazolone and acetic anhydride.—The pale yellow solution obtained on heating the substance with acetic anhydride, on being boiled for a quarter of an hour, gave a brownish yellow solid which crystallised from water, m.p. 295°.

The free base : 3-Amino-3 :5-endoxy-2 :3-dihydro-4 :1 :2-thiobiazole (III).—The acetyl derivative dissolved in gently boiling concentrated hydrochloric acid and deposited the hydrochloride on cooling; this crystallised from dilute hydrochloric acid, m.p. 107°, and gave the free base with sodium carbonate, m.p. 235° (Found : N, 36.15 ; S, 27.4. $C_2H_3ON_3S$ requires N, 35.9 ; S, 27.35 per cent.).

The identity of the acetyl derivative, the hydrochloride and the free base with the corresponding compounds of Guha (*loc. cit.*) was established. The free base gave compounds with phenyl *iso*cyanate m.p. 228° ; with phenyl mustard oil, m.p. 256° ; and with benzaldehyde m.p. 216°.

Isomer of phenylhydrazomonothiodicarbonamide (IV ; m.p. 200°).—Procedure adopted in the case of hydrazodithiodicarbonamide (part IV, 8) was followed. The crystalline solid obtained on cooling the solution of the hydrazide in 2*N* hydrochloric acid, was filtered, washed, and recrystallised from dilute alcohol, m.p. 175° (Found : N, 26.5 ; S, 15.1. $C_8H_{10}ON_4S$ requires N, 26.6 ; S, 15.25 per cent.).

Phenylhydrazomonothiodicarbonamide and concentrated hydrochloric acid (d. 1.19) : *Formation of 3-thiol-3 :5-endoxy-4-phenyl-2 :3-dihydro-4 :1 :2-triazole* (VII).—The hydrazide was boiled with the acid for fifteen minutes when from the clear solution a white solid began to separate ; it crystallised from dilute alcohol, m.p. 184°, is soluble in alkali, gives a disulphide with iodine, m.p. 196°, and a thiomethyl ether, m.p. 90-91° (Found : N, 21.9 ; S, 16.3. $C_8H_7ON_3S$ requires N, 21.75 ; S, 16.5 per cent.).

The *diacetyl* derivative crystallised from dilute alcohol, m.p. 213-214° (Found : N, 15.0 ; S, 11.3. $C_{12}H_{11}O_3N_3S$ requires N, 15.2 ; S, 11.5 per cent.).

3-Phenylamino-3 :5-endoxy-2 :3-dihydro-4 :1 :2-thiobiazole (VIII). —The above diacetyl derivative was boiled with concentrated hydrochloric acid and the cooled solution diluted with water when a crystalline white solid separated out slowly and was recrystallised from dilute alcohol, m.p. 206°. It is insoluble in cold dilute alkali, but on boiling the solution becomes pale green and liberates phenyl mustard oil. It is not desulphurised with mercuric oxide (Found : N, 21.95 ; S, 11.95. $C_8H_7ON_3S$ requires N, 21.7 ; S, 11.4 per cent.). It is identical with Guha and Chakraborti's compound obtained by the action of acetic anhydride directly on the hydrazide (*loc. cit.*).

Isomer of methylmonothiohydrazodicarbonamide (V ; m.p. 212°). —The isomeric hydrazide obtained by the action of 2*N* hydrochloric

acid melted at 168° (Found: N, 37.7; S, 21.45. $C_3H_8ON_4S$ requires N, 37.8; S, 21.6 per cent.).

3-*Thiol*-3:5-end*oxy*-4-*methyl*-4:1:2-*triazole* (IX).—The clear solution obtained by boiling the hydrazide with concentrated hydrochloric acid (d. 1.19) gave a solid on cooling which crystallised from dilute alcohol, m.p. 182°. It was soluble in cold dilute alkali, and with iodine gave a disulphide, m.p. 265° (Found: N, 31.6; S, 24.3. $C_3H_5ON_3S$ requires N, 32.06; S, 24.4 per cent.).

The *acetyl* derivative obtained from compound (IX) crystallised from dilute alcohol, m.p. 196-197° (Found: N, 24.05; S, 18.4. $C_5H_7O_2N_3S$ requires N, 24.3; S, 18.5 per cent.).

3-*Methylamino*-3:5-end*oxy*-2:3-*dihydro*-4:1:2-*thiobiazole* (X).—The above acetyl derivative with boiling concentrated hydrochloric acid (d. 1.19) gave on cooling a small quantity of a crystalline solid increased on concentration; it crystallised from aqueous alcohol, m.p. 232°, and was insoluble in cold dilute alkali (Found: N, 31.85; S, 24.45. $C_3H_5ON_3S$ requires N, 32.06; S, 24.4 per cent.).

Isomer of allylhydrazomonothiodicarbonamide (m.p. 190°; VI).—The isomeric hydrazide after crystallisation from alcohol melted at 192°. The difference between the melting points of the two isomeric hydrazides was only 2°, but a melting point of the mixture was 8-9° lower (Found: N, 32.42. $C_5H_{10}ON_4S$ requires N, 32.18 per cent.).

3-*Thiol*-3:5-end*oxy*-4-*allyl*-2:3-*dihydro*-4:1:2-*triazole* (XI).—The above hydrazide treated with concentrated hydrochloric acid as with the other hydrazides gave a product crystallising from dilute alkali; the disulphide melted at 174° (Found: N, 26.6; S, 20.3. $C_5H_7ON_3S$ requires N, 26.75; S, 20.4 per cent.).

The *acetyl* derivative from the triazole (X) and acetic anhydride had m.p. 171° (Found: N, 21.3; S, 15.74. $C_7H_9ON_3S$ requires N, 21.11; S, 16.08 per cent.).

3-*Allylamino*-3:5-end*oxy*-2:3-*dihydro*-4:1:2-*thiobiazole* (XII.)—The acetyl derivative was deacetylated by boiling concentrated hydrochloric acid, and the solution on concentration deposited the free *end*-oxytriazole as a white solid which crystallised from dilute alcohol, m.p. 210-211°. The substance is insoluble in cold dilute alkali (Found: N, 26.7; S, 20.32. $C_3H_7ON_3S$ requires N, 26.75; S, 20.4 per cent.).

SUMMARY.

The isomeric form of hydrazomonothiodicarbonamide could not be obtained, owing to ready decomposition ; but isomers of the substituted hydrazides have been isolated. Besides Freund's iminothiobiazolone, m.p. 177°, the unsubstituted hydrazide has yielded 3 :5-*end*-oxytriazole, m.p. 255° (I) and 3 :5-*endo*xy-3-aminothiobiazole, m.p. 235° (III) on treatment with hydrochloric acid of different concentration.

Hydrochloric acid (d.1.16 and 1.19) behaved similarly in effecting ring-closure of the mono-substituted hydrazides of this series, giving rise to *endo*xytriazole-thiols VII, IX and XI which in their turn were converted by acetylation followed by hydrolysis into *endo*xythiobiazoles VIII, X and XII by an interchange of position between the group NR of the nucleus and the sulphur atom.

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INDIAN MEDICINAL PLANTS.

PART I. WITHANIA SOMNIFERA

By D. N. Majumdar and P. C. Guha.

The plant *Withania somnifera*, Dunal (N.O., *Solanaceæ*) occurs in South Africa, South Europe, the West Coast of India and abundantly in Bengal. It is known in Sanskrit, Bengali and Telugu as Asvagandha ; English, Winter cherry ; Hindi, Asgandh ; Guzarati, Asundha ; Mahratta, Asagandha ; Tamil, Achuagandhi ; Canarese, Sogadeberu. It acts as a sedative and hypnotic (*Am. J. Pharm.*, 1891, **63**, 77 ; *Lancet*, 1886, **1**, 467) due to the presence of an alkaloid. It has been stated to be a tonic, alterative, aphrodisiac and nervine sedative by Nadkarni (Indian Materia Medica, pp. 900-903). Power and Salway (*J.C.S.*, 1911, **99**, 490-507) on analysing the root, leaves and stems of the S. African variety obtained potassium nitrate, tannin, colouring matters, glucose, phytosterol, hentriacontane, a mixture of saturated and unsaturated fatty acids, ipuranol, somnirol, somnitol, withanic acid, and a basic substance, probably an alkaloid.

It is not uncommon to find that the constituents of a plant differ with locality, and a preliminary examination revealed a wide discrepancy between the Bengal and S. African varieties of *Withania somnifera* (see table I). Investigation has shown that the indigenous herb contains the same non-basic constituents as the S. African variety, along with three basic materials not described by Power and Salway. Tonic and aphrodisiac properties of the Bengal variety are probably due to one of these.

EXPERIMENTAL.

The dried powdered material (25 g.) extracted with Prollius' fluid indicated the presence of alkaloids, and 50 g. successively extracted in a Soxhlet apparatus with various solvents gave the following amounts (grams) dried at 100°, from the two sources.

TABLE I.

	S. Africa	Bengal
Petroleum ether ... ... (b.p.50-60°)	0.22	0.33
Ether	0.35	0.43
Chloroform	0.23	0.25
Ethyl acetate	0.35	0.28
Alcohol	2.15	1.15
Total ...	3.30	2.44

The Bengal material (root, stems and leaves ; 50 lbs.) was extracted by continuous percolation with hot alcohol and after removing the solvent a dark coloured viscous mass (4 lbs.) with peculiar odour was obtained. The passage of steam gave no volatile product and there remained in the distillation vessel a dark solution and an insoluble, dark brown resinous mass (B) which weighed 275 gms. after drying, corresponding to about one per cent. of the weight of the herb.

The water-soluble constituents.—The solution was reduced to one fourth of its bulk by distillation under reduced pressure and extracted with (1) ether and (2) amyl alcohol. Both extracts gave after removal of the solvent small quantities of acid-soluble, brownish, viscous material which could not be crystallised.

The remaining aqueous liquid was treated with basic lead acetate solution while hot, when a voluminous greenish-yellow precipitate containing chiefly tannins and colouring matter was obtained. The filtrate was heated to boiling and freed from lead by sulphuretted hydrogen, the sweet, pale orange solution giving phenylglucosazone, m.p. 205°. The lead-free solution on concentration deposited some silica, and the filtrate from this on further concentration gave potassium nitrate (60 g.). Nothing definite could be isolated from the bitter, dark-coloured mother liquor remaining at this stage, which gave ammonia when boiled with alkali.

Insoluble resin, B.—The resin (275 g.) was powdered, made into paste with a small quantity of alcohol and thoroughly mixed with some alcohol-extracted residue of the drug. The solvent was removed by a current of air, and the resulting dry solid extracted successively with (1) petroleum ether, b.p. 50-60°, (2) ether, (3) chloroform, (4) ethyl acetate and (5) alcohol.

Petroleum extract of the resin.—The greenish, semi-solid viscous mass (35 g.) obtained after removing the solvent was dissolved in ether and shaken with concentrated hydrochloric acid which did not extract a basic substance. After being washed with water, the ether was removed and the semi-solid thus obtained heated with 2*N* alcoholic potash ; most of the alcohol having been evaporated, the product was mixed with filter paper pulp, evaporated to dryness and extracted with ether in a Soxhlet apparatus.

The unsaponifiable matter.—The residue (10 g.) from ether was boiled with excess of acetic anhydride on an oil bath, cooled and filtered. The insoluble portion crystallised from ethyl acetate in shining leaflets, m.p. 67-68°, thus agreeing with the melting point of hentriacontane (*J.C.S.*, 1911, **99**, 490-507). The acetic anhydride solution was poured into a large volume of water, neutralised with

sodium carbonate and extracted with chloroform. The solid left after the removal of chloroform was crystallised repeatedly from ethyl acetate, m.p. 125-28°, and on hydrolysis gave a white solid crystallising from ethyl acetate, m.p. 132-134°. It responds to the test for phytosterol and gives a precipitate with digitonin.

The fatty acid mixture.—The potassium salts left after ether extraction were dissolved in water, filtered, acidified with dilute sulphuric acid and extracted with chloroform, which deposited fatty acids (22 g.) comprising saturated (15 g.) and unsaturated (7.0 g.).

The unsaturated acids.—The light yellow oily mixture (iodine value 134.5; mean M.W. 282) was dissolved in glacial acetic acid (70 c.c.) and ether (140 c.c.) added; the pale yellow bromo-compound obtained with excess of bromine was fractionally crystallised from petroleum ether at low temperature (*Analyst,* 1898, 318). The first fraction (solid) was reduced with zinc filings in alcoholic solution, excess of zinc removed and the solution heated with dilute sulphuric acid (1 :10). The residue obtained from the ether extract of the solution was hydrolysed with alcoholic potash and after acidification extracted with ether which gave a liquid acid, iodine value 180-181; thus corresponding to linoleic acid. The second fraction (semi-solid) on being similarly treated gave a liquid acid, iodine value 90-91, and was oleic acid (*Arch. Pharm.*, 1919, **257**, 51).

The saturated acids.—The mixture of acids (4 g., mean M.W. 282.1; m.p. 68-72°) was dissolved in 94 per cent. alcohol (800 c.c.) and after 12 hours at 0° was filtered from the insoluble residue (IA); the filtrate reduced to half its bulk, was treated with an alcoholic solution of magnesium acetate (0.1204 g.) and left overnight. The acid liberated from the magnesium salt was purified by extraction with chloroform, mixed with (IA) and the mixture refluxed with 95 per cent. alcohol; the crystalline solid obtained therefrom on cooling was purified by repeated crystallisation from alcohol (m.p. 75-77°; mean M.W. 395.5). These results seem to indicate cerotic acid (m.p. 76-77°; M.W. 396.5). The solid obtained from the filtrate crystallised from alcohol, had m.p. 69-70° and mean M.W. 285; it was identified with stearic acid by an undepressed melting point when mixed with an authentic sample. The acid obtained from the alcoholic filtrate after magnesium acetate treatment and crystallisation from alcohol had m.p. 62-62.4° and mean M.W. 256; it was found to be palmitic acid (*cf.* Kries and Hafner, *Ber.*, 1903, **36**, 2766.)

Ether extract of the resin.—The greenish solid (about 20 g.) was digested with a large volume of ether, when a pale greenish amorphous mass (8.5 g.) remained undissolved; this on digestion with

hot alcohol left an insoluble residue (about 0.5 g.) crystallising from dilute pyridine in colourless, microscopic needles (0.3 g.) having m.p. 294-296° (decomp.). (Found: C, 72.3; H, 10.15. $C_{23}H_{40}O_4$ requires C, 72.6; H, 10.5 per cent.). The melting point of ipuranol recorded by Power and Salway is 290-300°.

From the above alcoholic solution withanic acid, m.p. 224-226°, somnirol, $C_{32}H_{43}O_6(OH)$, m.p. 203-4° and somnitol, $C_{33}H_{44}O_5(OH)_2$, m.p. 246-48° were isolated according to the method of Power and Salway.

The ethereal solution was extracted successively with 10 per cent. aqueous solutions of ammonium carbonate, sodium carbonate, sodium hydroxide and finally with concentrated hydrochloric acid. Nothing crystalline could be obtained from the brownish amorphous solid obtained from the alkaline extacts on acidification.

The acid extract on being carefully neutralised with sodium carbonate gave a light brown precipitate which did not melt sharply and could not be purified. It gave a dirty yellow precipitate (m.p. 156-157°; decomp.) with Meyer's reagent and a pale yellowish precipitate with gold chloride solution (m.p. 125-26°).

This amorphous basic substance was finally hydrolysed with alcoholic potassium hydroxide solution and extracted with ether, the ether extract shaken with dilute hydrochloric acid and the acid solution on being neutralised with sodium carbonate gave a white precipitate (1.0 g.) crystallising from alcohol in leaflets, m.p. 114.5-115.5° (Found: N, 14.89), evidently identical with the alkaloid, $C_{12}H_{16}N_2$ (requires N = 14.90 per cent.) described by Power and Salway (*loc. cit.*); but the material from which it was derived by alcoholic potash is different from their corresponding product (aurichloride, m.p. 185°). The ethereal solution remaining from these extractions contained green colouring matter (about 8 g.).

Chloroform extract of the resin.—The solid obtained by evaporating the solvent was heated with aqueous alcohol (5 per cent.) and dilute sulphuric acid for one hour on a water bath and filtered from the resin which was completely soluble in 10 per cent. sodium hydroxide solution; nothing definite could be obtained from this resin.

The acid extract on dilution with water gave a small quantity of yellowish amorphous material which could not be crystallised; m.p. 102°(decomp.); it does not appear to have been described by Power and Salway. The acid filtrate gave a precipitate (about 1 g.) on exact neutralisation with sodium hydroxide, insoluble in tartaric, but easily soluble in moderately dilute mineral acids. It was bitter, contained

nitrogen, but could not be crystallised; it gave with Meyer's reagent a dirty yellow, amorphous powder, m.p. 155-157°(decomp.), and thus appears to be identical with the alkaloid obtained from the ether extract.

The brown neutral solution had a bluish fluorescence and after acidification with hydrochloric acid gave a deep brown, amorphous precipitate with Meyer's reagent (m.p. 184-185.5° ; decomp.), which also does not appear to have been described by Power and Salway. It contained nitrogen and was not a glucoside, but the yield of material regenerated by sulphuretted hydrogen was insufficient for crystallisation and analysis.

Ethyl acetate extract of the resin.—The residue obtained from this extract was resinous and gave nothing definite.

Alcoholic extract of the resin.—The residue left after the removal of the solvent partly dissolved in dilute hydrochloric acid and the solution on basification yielded a small amount of a brownish amorphous solid containing nitrogen and responding to alkaloidal tests. The quantity of this basic substance was insufficient for purification.

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PART II. SWERTIA CHIRRETA.

By D. N. Majumdar and P. C. Guha.

Swertia Chirreta (N.O., Gentianaceae) grows abundantly in the temperate Himalayas from Kashmir to Bhutan and the Khasia range at altitudes from 4000 to 10,000 feet. The vernacular names are :—Charayatah (Hindi); Nila-vembu (Tamil); Kiratatikta chunimba (Sanskrit); Chireta (Bengali); Chiravata (Gujarati). Numerous other varieties of this drug are found in almost every part of India, and it has long been used by Hindu physicians as a bitter tonic, stomachic, febrifuge, antiperiodic, antibilious and anthelmintic, and in large doses as an aperient, the varieties differing in their strength (K. M. Nadkarni, Indian Materia Medica, p. 390). Experiments at the School of Tropical Medicine (Chopra, Ghosh and Ratnagiriswaran, *Ind. Jour. Med. Res.*, 1929, **16**, 770) show that it can effectively replace the gentian of the pharmacopoeia.

More than a century ago Richard Ratly separated from the drug a free acid, a very bitter extract, some resinous material and a large proportion of gums with potassium and calcium chlorides. Later, Höhn (*Jahr. Fort. Chem.*, 1869, 771; *Arch. Pharm.*, ii, **129**, 213) found the plant to contain a yellow, bitter acid, called ophelic acid and to which he assigned the formula, $C_{13}H_{20}O_{10}$ and another bitter principle chiratin, $C_{26}H_{48}O_{15}$, resolved with dilute hydrochloric acid into ophelic acid and chiratogenin, $C_{13}H_{24}O_3$. He obtained also a tasteless, yellow, crystalline substance in quantity too small for identification, but he did not record any melting points.

Examination of the drug has been undertaken (*a*) to make a more complete analysis of the constituents and (*b*) to inquire into the chemical nature of the active principles. Besides the foregoing compounds of Höhn, the following have now been isolated :—Palmitic, oleic and stearic acids, a phytosterol, a monohydroxy-acid, two yellow, crystalline phenols melting at 179-80° and 256-57° respectively, a neutral, pale yellow crystalline compound, m.p. 196-98° and a large proportion of resinous material. It is interesting to note that Höhn's chiratin does not appear to be a chemical individual, as two new substances melting respectively at 119° and 179-80° and a non-crystallisable viscous mass have now been isolated from the supposed chiratin.

Höhn suggested the presence of three double bonds in his formula, $C_{13}H_{20}O_{10}$, for ophelic acid; but from the composition of the bromine additive compound now prepared ($C_{13}H_{20}O_{10}Br_4$) it appears to contain only two double bonds.

EXPERIMENTAL.

The material employed in this investigation consisted of leaves, roots and stems of the plant obtained from the local market.

The dry and powdered drug (25 g.) extracted with Prollius' fluid having indicated absence of alkaloids, the material (50 g.) dried at 100° was extracted with various solvents in a Soxhlet apparatus, giving the following amounts (grams) dried at 100° :— Petroleum ether (b.p. 50-60°), 2.0; ethyl ether, 0.7; chloroform, 1.6; ethyl acetate, 0.92; ethyl alcohol (91 per cent.), 7.5; water, 8.0; total, 20.72.

For detailed investigation the disintegrated material (25 kg.) was thoroughly extracted by continuous percolation with 91 per cent. hot alcohol. Removal of the solvent left a dark, pungent, bitter viscous mass (about 2.75 kg.), some (1700 g.) of which was submitted to a current of steam to remove all volatile products. The turbid, pungent distillate was acidic, with minute drops of brownish oil on the surface. In the distilling flask there remained a dark-brown liquid (A) and a dark insoluble residue (B).

The steam distillate.—This was saturated with sodium chloride and extracted with ether, the slightly brownish extract being shaken successively with 10 per cent. sodium bicarbonate, sodium carbonate and sodium hydroxide. No definite chemical individual could be isolated from the resinous precipitates obtained from the alkaline extracts on acidification. The ethereal solution then left *iso*amyl alcohol (b.p. 129-131°; about 1.5 g.) while the residual aqueous solution on evaporation deposited a dark brown resinous mass from which nothing definite could be isolated.

Water-insoluble resin, B.—After repeated washing with hot water the dark, amorphous mass (700 g.) was extracted successively in a Soxhlet apparatus with solvents, when the following amounts (grams) of extracts dried at 80° were obtained.

Petroleum ether	(b.p. 50-60°)	145	20.7 per cent.
Ether	,,	55	7.85 ,, ,,
Chloroform	,,	39	5.57 ,, ,,
Ethyl acetate	,,	37	5.28 ,, ,,
Alcohol	,,	96	13.7 ,, ,,
	Total extract	372 g.	43.1 ,, ,,

The residue from the above extractions was an alkali-soluble greyish, resinous powder.

Petroleum ether extract.—The greenish semi-solid left after removal of the solvent was dissolved in excess of ether and successively extracted with 10 per cent. sodium bicarbonate, sodium carbonate and sodium hydroxide. No definite compound could be obtained from the first two, but the sodium hydroxide extract gave on acidification a greenish amorphous mass (about 1.4 g.) of which about 0.5 g. remained insoluble in 91 per cent. cold alcohol and crystallised from ethyl acetate in yellow needles with phenolic properties; yield, 0.3 g., m.p. 179-180° (Found: C, 61.9; H, 5.4. $(C_{13}H_{14}O_5)_x$ requires C, 62.4; H, 5.6 per cent.). The alcoholic solution on concentration and cooling gave a trace of the phenolic substance, but on complete evaporation, a brown resinous mass was obtained.

The washed ethereal solution after alkali extractions gave a viscous residue (about 142 g.) after removal of the solvent; this was saponified with an excess of 2*N* alcoholic potassium hydroxide and the unsaponifiable portion removed by ether extraction.

Saponification products.—The potassium salts left after ether extraction were dissolved in hot water and filtered from the filter paper pulp. The fatty acid mixture liberated from the aqueous solution on acidification was extracted with ether and the washed ethereal solution dehydrated with anhydrous sodium sulphate. After the removal of ether, the mixture of fatty acids was boiled in chloroform solution with animal charcoal for about two hours. The pale green filtrate gave a mixture of fatty acids (about 32 g.) from which the saturated (22 g.) and unsaturated (8 g.) acids were separated by Twitchell's method (*Ind. Eng. Chem.*, 1924, **13**, 806).

The unsaturated acids.—The yellowish oily acid after purification in the usual way through the sodium salt gave an iodine value, 90.5-91 and mean M.W. 281.3. It was oxidised with alkaline potassium permanganate (Le Sueur, *J. C. S.*, 1901, **79**, 1315) to a hydroxy-acid which on being repeatedly crystallised from alcohol formed colourless laminae, m.p. 126-127°, and was evidently dihydroxystearic acid. The unsaturated acid consisted, therefore, mainly of oleic acid (Found; C, 67.9; H, 11.8. $C_{18}H_{36}O_4$ requires C, 68.3; H, 11.4 per cent.).

The saturated acids.—The saturated mixture after purification gave a mean M.W. 298.3 and after being worked up according to the method of Kries and Hafner (*cf.* foregoing paper, p. 31) gave palmitic acid (m.p. 62.5°; mean M.W. 256.1), stearic acid (m.p. 69-69.5°; mean M.W. 284.4) and a third acid (m.p. 80-82°; mean M.W. 395.5) which appears to be cerotic acid. The identity of the first two acids with palmitic and stearic acids respectively has been confirmed by taking mixed melting points with authentic samples.

Unsaponifiable matter.—The ethereal extract of the saponified mass was washed with water, the residue from the solvent being then digested with 96 per cent. alcohol and filtered, but nothing definite could be isolated from the insoluble green viscous residue. The alcoholic solution after being boiled under reflux with animal charcoal for 3 hours was filtered hot and gradually treated with an excess of 1 per cent. hot solution of digitonin. After 12 hours in a cold chamber, the flocculent precipitate was boiled with xylene on a sand bath for 24 hours, when the filtered xylene was slowly evaporated to dryness. The residue, after several crystallisations from alcohol, formed colourless needles m.p. 128-130° ; it responded to the tests for phytosterol and gave an acetate, m.p. 127-29°.

Ether extract.—This was filtered from the less soluble portion (*a*, about 25 g.) which gradually separated during extraction. The filtrate gave an amorphous green powder (*b*, about 30 g.) after removal of the solvent. The less soluble portion (*a*) appeared to contain resinic acids from which no definite compound could be isolated. The green product (*b*) was partly (20 g.) insoluble in 60 per cent. alcohol, which extracted a very small amount of substance, m.p. 272-275° (decomp.) and a very bitter principle (about 2 g.) ; the former could not be identified due to insufficiency of material, and the latter on hydrolysis gave an unsaturated acid identified as ophelic acid.

The fraction insoluble in 60 per cent. alcohol was digested with a large excess of boiling 91 per cent. alcohol which dissolved almost all ; the filtrate on dilution with water after animal charcoal treatment gave a pale green precipitate which was repeatedly crystallised from alcohol, then dissolved in ether and shaken with a solution of sodium hydroxide, when the sparingly soluble sodium salt separated in the aqueous layer. The acid from the sodium salt crystallised from absolute alcohol in needles, m.p. 295°, is soluble in alcohol, ether, chloroform, and pyridine and burns without smoky flame (Found ; C, 77.3 ; H, 11.09. M.W. by Walker, Lumsden and McCoy's method, 395. $C_{26}H_{44}OH.COOH$ requires C, 77.51 ; H, 11.00 ; M.W. 418). The *acetyl* derivative crystallised from ethyl acetate in needles, m.p. 250-251° (Found : C, 76.49 ; H, 10.5 ; M.W. 442. $C_{29}H_{48}O_4$ requires C, 75.65 ; H, 10.04 ; M.W. 460).

Chloroform extract.—The less soluble portion (19 g.) which separated during extraction was found to be readily soluble in caustic alkali, but ethyl acetate extracted only 2 g., the remainder being resinous. The ethyl acetate solution on concentration after animal charcoal treatment gave a phenolic substance (about 1 g.) which after repeated crystallisation from ethyl acetate formed yellow needles, m.p. 256-57°. The portion soluble in chloroform and remaining on

evaporation was a dull yellowish, amorphous powder (20 g.) of which the alkali-soluble fraction was indefinite, while that insoluble in alkali yielded to acetone a small amount of neutral material which after repeated crystallisation from benzene formed pale yellow needles, m.p. 197-98° (Found C, 59.1; H, 6.27. $(C_6H_8O_3)_x$ requires C, 58.9; H, 6.5 per cent.).

Ethyl acetate and alcohol extracts.—Ethyl acetate and alcohol extracted respectively 37 g. and 19 g. of brown, alkali-soluble amorphous products from which nothing definite could be isolated.

The water-soluble portion, A.—The dark brown liquid was concentrated to a small volume, mixed with purified sawdust, dried and extracted successively with petroleum ether (b.p. 50-60°), ether and chloroform.

Nothing definite could be isolated from the petrol and the ether extracts. The latter, however, gave a small amount of a bitter substance which was found to be a mixture of ophelic acid (readily soluble in water) and chiratin (sparingly soluble in water). The latter was easily decomposed into ophelic acid and a tar by boiling, moderately dilute hydrochloric acid (see below).

Chloroform extract.—The very bitter residue from the solvent partly dissolved in cold water. The concentrated aqueous solution after alkali treatment was extracted with chloroform which gave a bitter, brownish material decomposing into ophelic acid on treatment with dilute acid. The alkaline solution after acidification was extracted with chloroform, but the viscous brown product therefrom could not be crystallised. It decolorises a dilute solution of potassium permanganate and bromine water, reduces Fehling's solution and alkaline copper sulphate, is bitter and dissolves in sodium bicarbonate solution with the evolution of carbon dioxide. An aqueous solution with excess of bromine gave a viscous, brown insoluble mass which was purified by repeated precipitation with water from an alcoholic solution as a pale brown, crystalline powder, m.p. 114-115.5° (Found: Br, 47.85. $C_{13}H_{22}O_{10}Br_4$ requires Br, 48.63 per cent.).

The water-insoluble, brown, viscous mass solidified during several months in a vacuum. Though soluble in alcohol, acetone and pyridine it could not be crystallised from these solvents. It is bitter, very hygroscopic and is hydrolysed by dilute hydrochloric acid into ophelic acid and a tar. From these properties it seems to be chiratin, the active principle of the drug.

The bitter principle.—The powdered dry plant material (100 g.) was exhausted with 63 per cent. hot alcohol, when the filtered solution deposited on cooling an amorphous, yellowish powder (0.6 g.) which

after several crystallisations from alcohol lost its bitter taste and became colourless, m.p. 268-70° (yield, 0.08 g.). The dry residue obtained from the cold filtrate on evaporation to dryness in presence of lead carbonate under reduced pressure was digested with cold water which dissolved ophelic acid. The chiratin was extracted by boiling alcohol from the residue as a dark yellowish, viscous material (1 g.) solidifying in a vacuum desiccator, representing 1 per cent. of the drug (Found: C, 50.99; H, 7.29. $C_{26}H_{48}O_{15}$ (Höhn) requires C, 50.00; H, 8.00 per cent.).

The dry material (Höhn's so-called chiratin) on digestion with ether partly dissolved leaving a mixture of a brown and pale yellow powder. The ether soluble portion on evaporation gave a greenish, viscous, bitter residue (0.4 g., i.e. 0.4 per cent. of the drug) and did not solidify even on keeping over sulphuric acid in a vacuum.

The ether insoluble residue partly dissolved in chloroform leaving a brown, crystalline powder which was washed thoroughly with chloroform and then dried in a vacuum desiccator; yield 0.4 g., i.e., 0.4 per cent of the drug. It begins to shrink at 145° and melts at 179-180° with decomposition, is intensely bitter and is insoluble in ether, petrol, alcohol and acetone, but soluble slightly in pyridine (Found: C, 50.02; H, 7.21. $(C_8H_{14}O_5)_x$ requires C, 50.5; H, 7.34 per cent.).

The chloroform solution gave an intensely bitter, greenish-yellow powder which melts at 118-119° and froths with decomposition at 121-122°; yield, about 0.1 g. i.e., 0.1 per cent. of the drug. It thus seems to be different from chiratin which according to Höhn (*loc. cit.*) is brown.

The cold aqueous solution was evaporated to dryness in a vacuum yielding a brown syrupy mass (1.2 g., i.e., 1.2 per cent of the drug). It gave the same bromo-compound, m.p. 114-116°, as ophelic acid described before.

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THE COLOURING CONSTITUENTS OF ALKANET ROOT (*ANCHUSA TINCTORIA*, LAM.).

Part I. The Constitution of Alkannin.

By Mangesh V. Betrabet and Gopâl Chandra Chakravarti.

Alkanet is well known as a red shrub growing in various parts of Europe and Asia. It was known to the Greeks and Romans as Anchusa, is mentioned in the works of Theophrastes and Pliny, and has continued to be regarded as a very valuable product during several centuries past. Its properties and uses are described in the Dictionaries of Chemistry by Watts and Thorpe and also in "The Natural Organic Colouring Matters" by Perkin and Everest.

The most important colouring matter of alkanet root, known as alkannin, has been examined by several chemists, but it is doubtful whether until very recently this dye has been obtained pure. Its composition has been given by various investigators as $C_{17}H_{10}O_4$ (Pelletier, *Annalen*, 1833, **6**, 27), $C_{35}H_{20}O_8$ (Bolley and Wydlers, *Annalen*, 1847 **62**, 41), $C_{15}H_{14}O_4$ (Carnelutti and Nasini, *Ber.*, 1880, **13**, 1514), $C_{15}H_{14}O_4$ or $C_{15}H_{12}O_4$ (Liebermann and Römer, *Ber.*, 1887, **20**, 2428) and $C_{30}H_{28}O_8$ (Gawalewski, *Chem. Zentr.*, 1902, **II**, 1001; from *Zeit. Oesterr. Apoth.*, 1902, V, **40**, 1001). Moreover, according to Liebermann and Römer it was found to give both anthracene and β-methylanthracene on distillation with zinc-dust. From these results Liebermann and Römer suggested that alkannin might be β-methyl-1 :4(or 1 :2)-dihydroxydi(or tetra)hydroanthraquinone, because of the ten known dihydroxyanthraquinones only the 1 :4- and 1 :2-dihydroxy-derivatives gave either blue or blue-violet colouration with alkali. Carnelutti and Nasini appear to have obtained a diacetyl-alkannin, $C_{15}H_{12}O_4(C_2H_3O)_2$, as a dull yellow micro-crystalline powder, and an indefinite barium compound containing two atoms of barium to three mols. of alkannin. Beyond this no information was available regarding the constitution of alkannin when the present investigation was undertaken.

A representative commercial sample of alkanet root was collected from the Indian bazaars for examination. The petrol extract of the root was subjected to an exhaustive purification, described in detail in the experimental part. Further purification through the acetyl derivative gave a reddish violet micro-crystalline product which was the

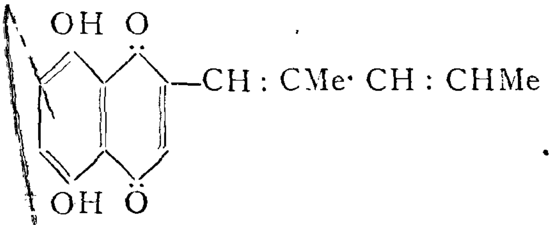
purest sample of alkannin that could be obtained, and analysis of this product gave the empirical formula, $C_{15}H_{13}O_4$. Several determinations of the molecular weight in camphor by Rast's method (*Ber.*, 1922, **53**, 1051) gave varying results, but pointed unmistakably to the molecular formula being double the empirical one. The percentages of barium and lead in the corresponding derivatives also supported the $C_{30}H_{26}O_8$ formula for alkannin confirmed by a molecular weight determination of acetylalkannin.

Having thus ascertained the molecular complexity of alkannin, we repeated the acetylation experiment of Carnelutti and Nasini (*loc. cit.*) and confirmed their result by isolating the acetyl derivative which, however, in the light of our previous observation should be properly represented as a tetra-acetyl derivative of the formula, $C_{30}H_{22}O_8(C_2H_3O)_4$. Benzoylation similarly gave a tetrabenzoyl derivative, $C_{30}H_{22}O_8(CO.C_6H_5)_4$, indicating the presence of four hydroxyl groups in alkannin. The nature of these groups was revealed by methylation, for while methyl iodide did not give definite products, dimethyl sulphate in alkali, which does not generally affect alcoholic groups, gave rise to a dimethoxyalkannin, $C_{30}H_{24}O_6(OMe)_2$, which could be benzoylated to a dimethoxydibenzoylalkannin, $C_{30}H_{22}O_6(OMe)_2((CO.C_6H_5)_2$. These results definitely showed that two of the hydroxyl groups were phenolic, and two alcoholic. It yielded also a dicarbethoxy-derivative, $C_{30}H_{24}O_6(O.CO_2Et)_2$ with ethyl chlorocarbonate. Bromination gave di-, tetra- and hexa-bromo-derivatives, $C_{30}H_{26}O_8Br_2$, $C_{30}H_{24}O_8Br_4$, and $C_{30}H_{22}O_8Br_6$. Bromination of the tetra-acetyl derivative gave tetra-acetyltetrabromo-alkannin, $C_{30}H_{20}O_8Br_4$, $(CO.CH_3)_4$. Alkannin is not oxidised by hydrogen peroxide. Chromic acid and permanganate oxidised it completely and no definite oxidation product could be isolated. Fuming nitric acid also acted very energetically, oxalic and succinic acids being isolated from the mixture together with a nitro-compound, m.p. 287°. Oxidation with dilute nitric acid gave oxalic acid and two derivatives, one containing nitrogen and melting at 297°, the other a nitrogen-free neutral substance, m.p. 320°. Distillation with zinc-dust gave β-methylanthracene. Tetra-acetylalkannin, on treatment with fuming nitric acid, yielded a dinitrotetra-acetylalkannin.

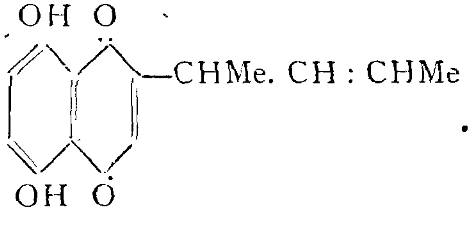
The above results were communicated to the Indian Science Congress in two papers in September 1929 and 1930 and read in the subsequent meetings of the Congress (*Proc. Ind. Sci. Cong.*, 1930, p. 181 ; 1931, p. 166). Meanwhile we were studying several complex nitro-carboxylic acids obtained from alkannin with dilute nitric acid when papers appeared in the *Berichte* on the constitution of alkannin. It therefore seems desirable to place the results of our investigation on

record without further delay and reserve the oxidation experiments for a further communication.

Raudnitz, Redlich and Fiedler (*Ber.*, 1931, **64**, 1835) purified alkannin from a sample of alkannin reagent supplied by E. Merck by precipitating it from alkaline solution and then repeatedly precipitating it from benzene solution by petrol, and found the empirical formula $C_{16}H_{14}O_4$. They apparently obtained a tetra-acetyl leuco-alkannin, $C_{16}H_{12}O_4(CO.CH_3)_4$, and ascertained the molecular weight in camphor. On distillation *in vacuo* it gave a small quantity of naphthazarin. Hydrogenation of the acetyl leuco-compound pointed to a side chain with two ethylenic bonds. Basing their arguments on these meagre details they provisionally suggested the following constitution for alkannin,



Two months later, however, Dieterle, Salomon and Nosseck (*Ber.*, 1931, **64**, 2086) claimed to have obtained pure alkannin corresponding to the formula, $C_{15}H_{14}O_4$ confirmed by the analysis of a diacetate and a dihydroalkannin tetra-acetate. Distillation of alkannin with zinc-dust yielded β-methylanthracene. Ozonisation in chloroform yielded a compound, $C_{13}H_8O_8$, regarded as a dihydroxynaphthaquinonedicarboxylic acid, which lost carbon dioxide on heating. They observed that the behaviour of alkannin towards ozone differed from that of synthetic 5 :8-dihydroxy-1-methyl-1 :2 :3 :4-tetrahydroanthraquinone and 5 :8-dihydroxy-2-methyl-1 :2 :3 :4-tetrahydroanthraquinone. They therefore concluded that alkannin could not be any of these reduced anthracene derivatives, and that probably its reactions might be best explained by the formula,

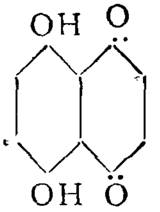


Raudnitz, however, in a later paper (*Ber.*, 1932, **65**, 159) confirms the composition, $C_{16}H_{14}O_4$, by repeating the preparation and analysis of

acetyl leuco-alkannin. He shows that the product of sublimation of alkannin is naphthazarin, not 1-methylquinazarin as supposed by Dieterle, Salomon and Nosseck. He considers it improbable that under the conditions employed by Dieterle, Salomon and Nosseck they obtained 1-methyl- and 2-methyltetrahydroquinazarin.

It is significant that the above investigators do not mention the work of Gawalewski (*loc. cit.*) who arrived at the formula, $C_{30}H_{28}O_8$, for alkannin. It is not probable that Raudnitz, Redlich and Fiedler could obtain pure alkannin by the method they utilized, because we have observed that the waxy material associated with the colouring matter is held so tenaciously that it cannot be removed completely by extracting the dye with alkalis. Moreover the wax and the fatty acids derived from it are all very sparingly soluble in petrol and very rich in carbon, so that even a moderate contamination with any of them may account for the slight increase in the carbon content observed by Raudnitz and his co-workers.

On the other hand, absence of data regarding the molecular weight of alkannin in the experiments of Dieterle, Salomon and Nosseck appears to have caused this confusion regarding its constitution. We consider that a formula satisfactorily explaining the properties of alkannin must embrace the facts that (i) the molecular formula is double the empirical formula, (ii) β-methylanthracene is one of the degradation products, (iii) naphthazarin and β-naphthylacetic acid should form part of the skeleton of alkannin, and (iv) the formula should contain two phenolic and two alcoholic groups. It is no doubt very difficult to represent so complex a structure as $C_{30}H_{26}O_8$ without careful examination of a greater number of its degradation products, but the results available provide a formula tentatively explaining all the known facts concerning alkannin. We believe that associating a reduced β-methylanthraquinone with the formula suggested by Dieterle and his coworkers will be the most satisfactory procedure. The production of naphthazarin by vacuum sublimation and of β-methylanthracene by zinc-dust distillation indicates the following two basic structures in alkannin :



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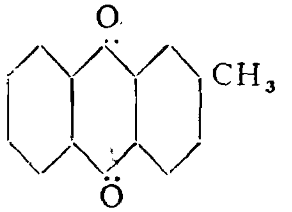
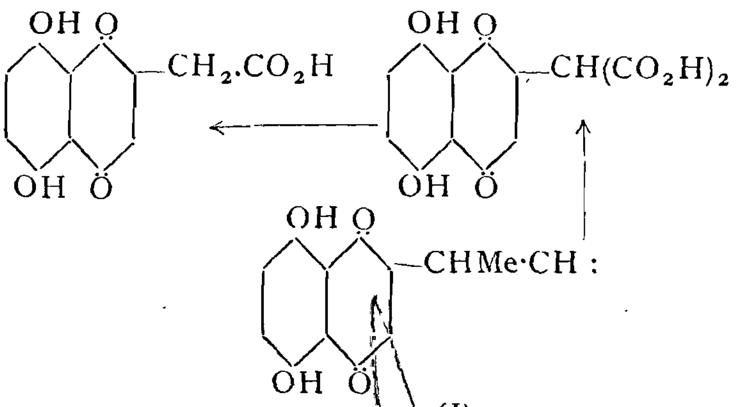


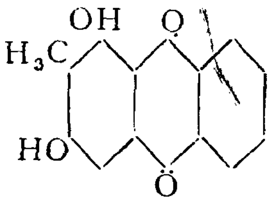
The ozonisation experiment of Dieterle, Salomon and Nosseck, yielding a dihydroxynaphthaquinonedicarboxylic acid which becomes

a monocarboxylic acid with loss of carbon dioxide, indicates a side chain of the following type (I) attached to the naphthalene ring



(I)

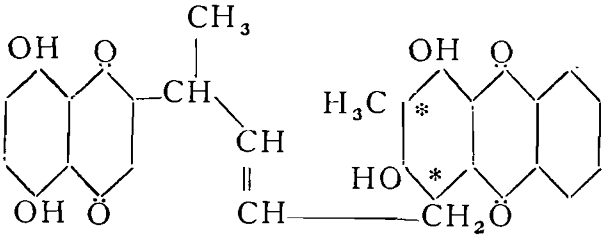
It may be safely assumed that the two non-phenolic hydroxyl groups exist in the anthraquinone part of the molecule. In accordance with some interesting generalisations made by Mitter (*J. Ind. Chem. Soc.*, 1930, **7**, 40) and Mitter and Biswas (*ibid.*, 1928, **5**, 769) from a study of numerous anthraquinone derivatives occurring in nature, we suppose that a dihydroxy-β-methylanthraquinone will have one of the hydroxyl groups in the β-position and the other in the α-position. The structure of the anthraquinone part of alkannin therefore becomes



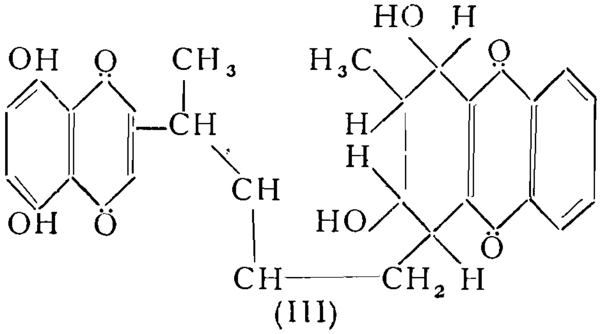
(II)

which accounts for fifteen carbon atoms. If two carbon atoms are added to the side chain of the naphthalene ring (I) we obtain the formula of Dieterle, Salomon and Nosseck (see above) which accounts for the remaining carbon atoms. Two hydrogen atoms will be utilised by a

combination of the two above formulae which may be represented by a structure of the type



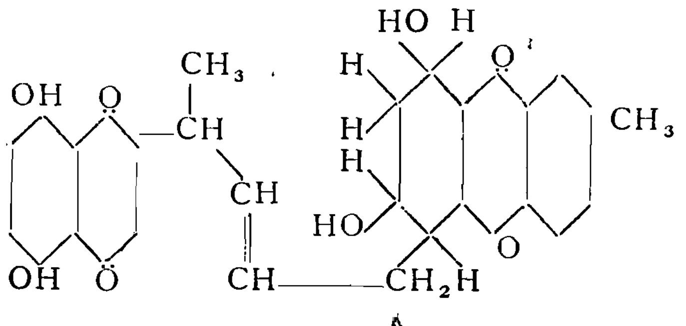
where the positions of the methyl group and the side chain indicated by stars are somewhat uncertain. However, as it happens that in a tetra-substituted anthraquinone derived from (II), the third hydroxyl group takes up the α-position we presume that this is the next reactive position and the link with the side chain is established here, possibly by the elimination of this hydroxyl group as water. The detection of traces of succinic acid in the oxidation products of alkannin by Liebermann and Römer and by us, is strong evidence in favour of this conclusion. The alcoholic character of the two hydroxyl groups in the anthraquinone part would be explained if the ring containing them is reduced, so that the molecule of alkannin becomes $C_{30}H_{26}O_8$ and is finally represented by the formula (III)



(III)

The possibility of the methyl group in the β-position being present in the other benzene nucleus necessitating the modification of the above formula as below is likewise excluded from a consideration of the homonuclear arrangement of the hydroxyl groups (*cf.* Mitter and Biswas, *loc. cit.*).

This constitution (III) harmonises with the chemical behaviour of alkannin and explains the spectroscopic examination, in which its bands



differ so much from the absorption bands given by either naphthazarin or the hydroxyanthraquinones.

It is expected that investigation of degradation products from alkannin, combined with examination of the associated colouring matters in the alkanet root, will throw further light on the constitution of alkannin. Experiments on these lines are in progress.

EXPERIMENTAL.

Extraction of Alkanet Root.—The powdered root (4 lbs.) was extracted with petrol (b.p. 50-60°) in a modified Soxhlet apparatus (Sando, *Ind. Eng. Chem.*, 1924, **16**, 1125) till the final extract was colourless. The petrol was distilled, the residue dissolved in chloroform was filtered, and the viscous mass left by the chloroform was extracted with cold acetone, in which a white waxy mass remained undissolved. After removing acetone, the residue was repeatedly extracted with 5 per cent. sodium hydroxide solution, which left a blackish violet powder undissolved. The intense blue alkaline solution was filtered first through muslin cloth and then through folded filter paper, almost neutralised with acetic acid and completely with dilute hydrochloric acid. The precipitated sticky mass was twice more extracted with 3 per cent. sodium hydroxide solution and acidified, the resulting solid being extracted with benzene in a Soxhlet apparatus. On removing the solvent, alkannin remained as a dark-red powder completely soluble in alkali. Yield 1.2-1.5 per cent.

Tetra-acetylalkannin.—Alkannin (50 g.) with fused sodium acetate (15 g.) and excess of acetic anhydride was heated under reflux during 5 hours at 140°. The product was poured into excess of water, neutralised with sodium carbonate, and the solid after extraction with

alcohol was crystallised from acetic acid, forming brown micro-crystals. It decomposes at a high temperature, is soluble in ethyl acetate, acetone and acetic acid, but is insoluble in methyl, ethyl and amyl alcohols, ether, benzene etc. Yield 80-85 per cent. (Found : C, 66.58 ; H, 5.23 ; COMe, 24.5 ; M.W. (Rast's method), 687. $C_{38}H_{34}O_{12}$ requires C, 66.86 ; H, 4.99 ; COMe, 25.2 ; M.W., 682).

Alkannin.—Tetra-acetylalkannin (50 g.) was hydrolysed with caustic potash (40 g.) in methyl alcohol ; poured into water, filtered and acidified. The red flocculent precipitate was dissolved in glacial acetic acid, the solution concentrated, treated while hot with a drop or two of water and allowed to stand. This process was repeated twice, when alkannin separated as a violet-red micro-crystalline powder which shrinks at 180° and decomposes with swelling at 220° ; at higher temperatures it becomes a carbonaceous mass (Found : C, 69.74 ; H, 5.38 ; M.W. (Rast's method), 510, 521, 525, 537. $C_{30}H_{26}O_8$ requires C, 70.0 ; H, 5.05 per cent. M.W., 514). Yield, 25-30 g.

Methylation.—Alkannin (5 g.) was dissolved in caustic potash (12 g. in 15 c.c. of water) and treated with dimethyl sulphate (15 c.c.) in small quantities with vigorous shaking. The same quantities of methyl sulphate and alkali were again added, the alkaline solution heated under reflux during three hours, acidified, the product again treated with alkali and methyl sulphate as before and finally boiled for an hour and filtered. The solid was washed with hot dilute alkali and water, dried, extracted with chloroform and the solvent distilled. *Dimethoxyalkannin* separated from dilute acetic acid in brown micro-crystals (Found : C, 70.46 ; H, 5.85 ; OCH_3, 12.2. $C_{30}H_{24}O_6(OMe)_2$ requires C, 70.84 ; H, 5.53 ; OMe, 11.44 per cent.). Raudnitz, Redlich and Fiedler failed to methylate alkannin by diazomethane and methyl *p*-tolylsulphonate.

Benzoylation.—Benzoyl chloride was gradually added to a pyridine solution of alkannin with stirring and cooling ; the mixture was then heated on a water-bath during one hour and poured into dilute hydrochloric acid. The sticky mass gradually solidified and separated from alcohol in pale brown micro-crystals. *Tetrabenzoylalkannin* decomposes above 260° (Found : C, 74.51 ; H, 4.94. $C_{30}H_{22}O_8(CO.C_6H_5)_4$ requires C, 74.8 ; H, 4.52 per cent.). Carnelutti and Nasini (*loc. cit.*) state that benzoyl chloride does not react with alkannin.

The *barium* compound.—Alkannin dissolved in absolute alcohol was treated with an ammoniacal solution of barium chloride. The blue precipitate was filtered, and washed thoroughly with water, alcohol and ether (Found : Ba, 12.27. $(C_{30}H_{25}O_8)_2$ Ba requires Ba, 11.78 per cent.).

The *lead* compound was obtained by adding lead chloride to the solution of the potassium derivative of alkannin, prepared from alcoholic potash and an alcoholic solution of alkannin (Found : Pb, 29.34. $(C_{30}H_{25}O_8)_2Pb_2$ requires Pb, 28.75 per cent.).

Dimethoxydibenzoylalkannin.—Dimethoxyalkannin was benzoylated in pyridine, the product after preliminary purification separating from acetic acid in brown micro-crystals (Found : C, 72.9 ; H, 5.31. $C_{46}H_{38}O_{10}$ requires C, 73.6 ; H, 5.07 per cent.).

Dicarbethoxyalkannin.—Alkannin (1 g.) dissolved in pyridine (8 c.c.) was treated with ethyl chlorocarbonate (5 g.) in the cold and the mixture warmed on a water-bath during two hours. On pouring into dilute hydrochloric acid the carbethoxy-compound separated as a blackish brown mass which crystallised from dilute acetic acid in deep brown needles. It does not melt, but decomposes above 270°. The compound can be easily hydrolysed with dilute alkalis (Found C, 65.31 ; H, 5.42. $C_{30}H_{24}O_6(O.CO_2Et)_2$ requires C, 65.6 ; H, 5.17 per cent.).

Bromination.—Alkannin (2 g.) was placed on a watch glass over which an open dish containing dry bromine (excess) was kept in a desiccator during four days. The viscous product was heated on the water-bath to expel occluded hydrobromic acid, washed with alcohol, dissolved in ethyl acetate and filtered into excess of petrol. This process was repeated three times, the finally violet-brown precipitate separating from acetic acid in deep brick-red micro-crystals. *Hexabromoalkannin* decomposes on heating (Found : Br, 48.36. $C_{30}H_{22}O_8Br_6$ requires Br, 48.48 per cent.).

Bromination in cold acetic acid.—Bromine (6 mols.) in acetic acid was added slowly with shaking and under ice-cooling to alkannin (2 g.) dissolved in acetic acid (10 c.c.) ; after 12 hours at the room temperature, the mixture was poured into water, filtered and washed well with hot water. A portion of the precipitate dissolved in ether. *Tetrabromoalkannin* was obtained from acetic acid as a brick-red powder blackening at 260° (Found : Br, 38.12. $C_{30}H_{24}O_8Br_4$ requires Br, 38.46 per cent.). The ether-insoluble portion was *dibromoalkannin*, crystallising from acetic acid in red aggregates (Found : Br, 23.55. $C_{30}H_{26}O_8Br_2$ requires Br, 23.74 per cent.).

Bromination in hot acetic acid.—The experiment was conducted as before, but the mixture was heated on a water-bath during three hours and then poured into water. The precipitate, after extraction with ether and alcohol, crystallised from acetic acid in deep brick-red aggregates, and was hexabromoalkannin (Found : Br, 48.21. $C_{30}H_{22}O_8Br_6$ requires Br, 48.48 per cent.).

Bromination of tetra-acetylalkannin.—The acetyl compound (3 g.) was dissolved in acetic acid (10 c.c.) and shaken with an acetic acid solution of bromine (4 mols.) added in small quantities at a time. The mixture was heated on a water-bath during one hour, cooled and poured into water, when a brownish precipitate separated. After extraction with carbon disulphide the residue was dissolved in acetic acid from which one drop of water precipitated *tetra-acetyltetrabromoalkannin* as a brown, granular mass. For complete purification, the above process had to be repeated three times. The compound decomposes at about 280° with blackening (Found : Br, 31.82. $C_{30}H_{20}O_8Br_4(C_2H_3O)_4$ requires Br, 32.0 per cent.).

Oxidation with fuming nitric acid.—Alkannin (5 g.) was gradually added to nitric acid (15 c.c., d. 1.5) at 0°. After an hour's stirring the mixture was allowed slowly to attain the room-temperature when oxides of nitrogen were freely evolved. Action having ceased, the mixture was poured on crushed ice, filtered and washed well with ice-water which dissolved a large proportion. The insoluble residue separated from alcohol in reddish brown micro-crystals, m.p. 287° with explosion ; it contains nitrogen and is under investigation. The filtrate on evaporation to dryness deposited a pasty mass which on extraction with ether left a powder crystallising from alcohol in colourless prismatic needles identified as oxalic acid (m.p. 101°). No definite compound could be isolated from the ether extract.

Oxidation with dilute nitric acid.—Alkannin (5 g.) was added to nitric acid (100 c.c., d. 1.2) shaken well during one hour and then slowly heated on a water-bath during 48 hours, fairly vigorous action with frothing taking place at first ; after 24 hours the solution became yellow. Dilution gave an orange nitro-compound which separated from acetic acid in orange-yellow prismatic crystals, m.p. 297° (decomp.). The filtrate on evaporation and extraction with ether left a white powder which was washed with alkali ; it crystallised from alcohol in colourless needles, m.p. 320°, and did not contain nitrogen, while oxalic acid was isolated from the alkaline solution. The ethereal extract was evaporated, and a solution of the residue in alcohol was boiled with animal charcoal ; from the filtrate when kept in a vacuum during several days there separated a few crystals which on recrystallisation melted at 178°, corresponding nearly to succinic acid.

Action of fuming nitric acid on tetra-acetylalkannin.—Tetra-acetylalkannin (2 g.) dissolved in acetic anhydride (5 c.c.) was gradually added to a mixture of nitric acid (3 c.c., d. 1.5) and acetic anhydride (8 c.c.) under cooling. After 12 hours the mixture was poured into water, the resulting reddish brown solid separating from acetic acid as a brown micro-crystalline powder. The *dinitrotetra-acetylalkannin* does

not melt, but blackens at 260° (Found: C, 59.12; H, 4.61; N, 3.38. $C_{38}H_{32}O_{16}N_2$ requires C, 59.07; H, 4.15; N, 3.62 per cent.).

Distillation of alkannin with zinc-dust.—A mixture of alkannin and zinc-dust in equal quantities was distilled under reduced pressure, yielding a yellowish oil which solidified slowly; the product was dissolved in benzene, washed with sodium carbonate and hydroxide solutions and freed from solvent. The residue was extracted with alcohol which on concentration gave a few crystals of β-methylanthracene, m.p. 198-200°.

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II. NOTE ON CONSTITUENTS OF THE WAX FROM ALKANET ROOT.

By M. V. Betrabet and G. C. Chakravarti.

The roots of alkanet are known to contain a colourless wax which is extracted by petrol along with the colouring matter, alkannin. Liebermann and Römer (*Ber.*, 1887, **20**, 2428) first mentioned it as a colourless waxy substance, m.p. 76°, which on prolonged treatment with alkali gave an inert compound and an acid. As alkannin is a fat-soluble dye exceedingly sparingly soluble in petrol, and the wax is slightly more soluble, it is considerably more convenient to extract and purify the former. But the wax is not present in quantity sufficient to enable the whole of the alkannin to be extracted by petrol, a considerable amount of this dye remaining in the root with anchusin, the second colouring matter of alkanet, to be described in a later communication. A preliminary examination of the wax shows that it is principally an ester of carnaubyl alcohol and cerotic acid.

EXPERIMENTAL.

The petrol extract of powdered alkanet root was evaporated to dryness and the residue treated with cold acetone, when a white wax tinged with red remained ; the crude wax was dissolved in methyl ethyl ketone and boiled with animal charcoal during four hours. The filtrate on concentration gave apparently crystalline flakes, recrystallising from ethyl acetate in white flakes m.p. 78° (Found : C, 81.95 ; H, 13.51 per cent.). The wax (yield, 0.18-0.2 per cent.) gave the following constants :—

$d^{30°}$	0.9676
$n_D^{80°}$	1.438
Saponification value	69.16
Acid value	12.92
Unsaponifiable matter	58.56
Iodine value	12.2
Molecular weight of mixed acids ...	380

The wax was saponified with an alcoholic solution of sodium hydroxide, the soap extracted with ether to remove unsaponifiable matter and the acids regenerated from the salts by acidification with hydrochloric acid. Two acids were isolated : (*a*) m.p. 68° (very small

quantity) ; (*b*) cerotic acid, m.p. 76° with equivalent, 393.4 ; $C_{26}H_{52}O_2$ requires 396.

Unsaponifiable Matter.—The ether extract from the sodium salts was evaporated to dryness and the residue crystallised from alcohol several times. The m.p. 68° indicates carnaubyl alcohol (Found : C, 81.28 ; H, 14.2. $C_{24}H_{49}$.OH requires C, 81.35 ; H, 14.12 per cent.). The alcohol was oxidised with chromic acid in acetic acid solution, the white product crystallising from ether and melting at 72° in agreement with carnaubic acid (Found : C, 78.33 ; H, 13.16. $C_{24}H_{48}O_2$ requires C, 78.26 ; H, 13.05 per cent.).

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THE VELOCITY OF ALCOHOLYSIS OF ESTERS OF DIBASIC ACIDS.

By Z. H. Patel and H. E. Watson

The velocity of alcoholysis of several esters of monobasic acids has been measured by Kolhatkar (*J.C.S.*, 1915, **107**, 921) and by Dasannacharya and Sudborough (*J. Indian Inst. Sci.*, 1921, **4**, 81; 1924, **7**, 1) but no corresponding observations appear to have been made with esters of dibasic acids, although the interchange of radicals has been demonstrated. Leuchs and Theodoresen (*Ber.*, 1910, **43**, 1239) examined the conversion of diethyl malonate into the methyl ester in presence of ammonia; Bruni and Contardi (*Atti R. Accad. Lincei*, 1906, V, **15**, 637; Gazzetta, 1906, **36**, 356) showed by polarimetric measurements that menthol reacts with ethyl oxalate and malonate with appreciable velocity at 100°, while Pfannl (*Monatsh.*, 1910, **31**, 307) taking advantage of the insolubility of certain esters showed that the rate of interchange of methyl, ethyl and propyl radicals in the esters of terephthalic, benzoic and oxalic acids was approximately proportional to the amount of alkali added as a catalyst.

In the case of an ester with two replaceable groups, the question arises whether both groups are replaced simultaneously or successively and in the latter case whether the velocity of alcoholysis of each group is the same. A reaction in which two alkyl groups simultaneously react with one molecule of an ester is most improbable, while the formation of an intermediate compound has been proved definitely by Sudborough and Bhagvat for the case of methyl oxalate and *iso*butyl alcohol (*J. Indian Inst. Sci.*, 1920, **2**, 121). It thus seems certain that the reaction takes place in two stages. If this is so, it is evident that in the most general case four reactions occur. Consider the action between ethyl alcohol and the ester $COOMe.R_1......R_2\,COOMe$. Either of the methyl groups may react giving rise to the compounds $COOEt.R_1......R_2.COOMe$ and $COOMe.R_1......R_2.COOEt$, each of which can react with another molecule of ethyl alcohol. The equation representing the velocity of these reactions is one of some complexity but it becomes very much simplified if it is assumed that the substitution of Me by Et in the grouping attached to R_2 does not alter the rate of reaction of the other Me group, or in other words, if the velocity of esterification of the Me group adjacent to R_1 in the esters $COOMe.R_1...R_2.COOMe$ and $COOMe.R_1....R_2.COOEt$ is the same. With this assumption the case becomes that of the esterification of a

mixture of the esters $R_3.COOMe$ and $R_4.COOMe$ in equimolecular proportion. If a large excess of alcohol is used, both reactions may be regarded as unimolecular and the following equations hold good.

$$a - x_1 = ae^{-K_1 t} \qquad a - x_2 = ae^{-K_2 t} \qquad (1)$$

where x_1 x_2 are the concentrations of ester formed after time t, a is the initial concentration and K_1 K_2 are the velocity coefficients. Corresponding with every molecule esterified there will be a definite volume change. If this is assumed to be constant so that the total volume change is proportional to x, putting $x = bv$ and $a = bV$ where V is the volume change when the reaction is complete,

$$V_1 - v_1 = V_1 \, e^{-K_1 t} \text{ and } V_2 - v_2 = V_2 \, e^{-K_2 t} \qquad (2)$$

The total volume change at time t is—

$$v_1 + v_2 = V_1 \, (1 - e^{-K_1 t}) + V_2 \, (1 - e^{-K_2 t})$$

No data are available for determining V_1 and V_2, but if they are equal

$$v/V = 1 - \tfrac{1}{2} \, (e^{-K_1 t} + e^{-K_2 t}) \qquad (3)$$

In the case of a symmetrical ester $K_1 = K_2$ and $v/V = 1 - e^{-Kt}$ or

$$\ln \, (1 - v/V) = Kt \qquad (4)$$

the usual equation for a unimolecular reaction.

The reaction velocity for several methyl and ethyl esters of dibasic acids in presence of hydrochloric acid has now been measured by a dilatometric method and the velocity coefficients calculated by means of equations (3) and (4). The extent to which the assumptions made in deducing these equations are justified may be seen from the results which follow.

EXPERIMENTAL

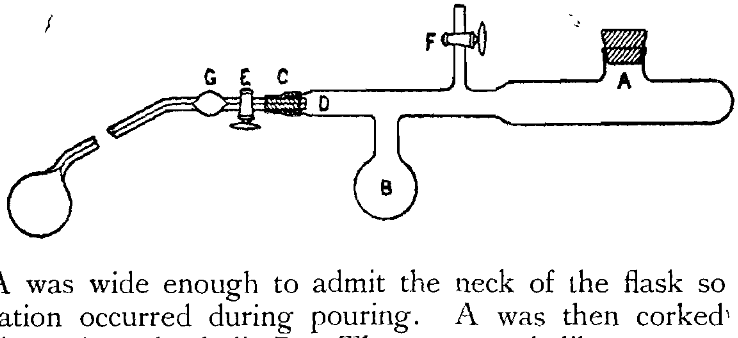
Preparation of esters.—The esters were prepared by refluxing the acids with 5 molecular proportions of absolute alcohol containing 3 to 4 per cent of hydrochloric or sulphuric acid, except in the case of oxalic acid for which no catalyst was required. After separation and neutralisation they were dried with anhydrous magnesium sulphate and fractionated under reduced pressure. All boiled within a range of/1° and the physical constants agreed with those given in the literature. Methyl azelate which does not appear to have been prepared previously was made from a pure specimen of azelaic acid; it boiled at 175° (unc.) /38mm,

Methyl and ethyl alcohols after preliminary drying were treated 3 times with metallic calcium and fractionated. Their densities showed that they contained less than 0.03 per cent of water. They were stored in Jena glass bottles with syphon delivery tubes connected to burettes and protected from moisture by means of soda lime and phosphorus pentoxide.

Preparation of the reaction mixtures.—The presence of water even in small quantities is known to exert a very great influence upon reactions involving alcoholysis. No actual measurements were made with esters of dibasic acids, but it has been found during the course of analogous experiments not yet published that the addition of 1 per cent. of water reduces the velocity coefficient of alcoholysis of methyl propionate by 60 per cent. It was necessary therefore to take very great care not to expose the solutions to the atmosphere at any time. This was effected by running the necessary quantity of absolute alcohol from its burette into a Jena glass bottle filled with dry air and fitted with a two-holed cork to which a drying tube was attached. The tip of the burette was withdrawn from the second hole and a glass tube inserted, through which dry hydrogen chloride from fused ammonium chloride and sulphuric acid could be passed into the alcohol. By counting the bubbles of gas in a sulphuric acid bubbler it was possible to prepare a solution of suitable strength which was approximately double the concentration used for alcoholysis. A sample of the acid solution was withdrawn and the exact strength found by titration. The remaining solution was then syphoned into a dry burette and the required quantity run into a 50 c.c. graduated flask containing a measured quantity (2-3 c.c.) of ester protected from moist air and the mixture diluted to 50 c.c. with alcohol.

To fill the dilatometer, the flask after shaking was emptied into the special apparatus (shown in fig. I) previously filled with dry air. The

Fig. I



T-tube A was wide enough to admit the neck of the flask so that no contamination occurred during pouring. A was then corked and all the liquid run into the bulb B. The evacuated dilatometer was then inserted through the small rubber tube C which up to this stage had

been closed with a glass rod. The liquid was poured into the lower portion D of the filler and the dilatometer filled as much as possible by opening the tap E. Removal of the last bubbles of air was effected by evacuation of the apparatus through the tap F and readmission of dry air. The bulb G greatly facilitated the filling operation which could be carried out very quickly in practice. The final adjustment of the liquid in the capillary to a suitable level was made in the thermostat. A trial experiment made by breaking a dilatometer bulb and titrating the contents, showed that the concentration of acid was unchanged by this process of filling.

The dilatometers had a volume of about 40 c.c. and the capillaries, bent as recommended by Kolhatkar (*loc. cit.*) to facilitate clamping, had a cross section of 0.0012 to 0.0018 sq. cm. The bore and uniformity were measured by weighing threads of mercury. All experiments were made in a thermostat at 30° but as a difference of temperature of 0.01° caused a movement of the meniscus of 3 mm., a second bulb containing alcohol and acid but no ester was used as a blank to allow for small temperature variations and also for small changes in volume which might occur owing to the action of the acid on the alcohol when methyl alcohol was used. The dilatometer tap was kept closed except during a reading when it was opened to equalise the pressure, and the position of the meniscus was ascertained by measuring its distance from a fixed mark on the dilatometer stem with a cathetometer.

RESULTS

In Table I detailed readings for one experiment are given in order to illustrate the method of calculation. Since the ester was symmetrical, the velocity coefficients K were calculated by means of equation (4) and the results were constant to the limit of experimental error. The total change in volume has been assumed to be the change corresponding with the completion of the reaction. Strictly speaking it corresponds with the equilibrium point, but with the large excess of alcohol used the correction to K is small, amounting in the present case to about 2.8 per cent. The application of this correction is discussed in greater detail on p. 64. The dilatometer readings were corrected for variations in the volume of liquid in the bulb used as a blank by adding or subtracting the observed change (in mm.) multiplied by a factor, 0.8, which allowed for the difference in volumes of the bulbs and capillaries. In most cases experiments were conducted with two different strengths of acid and the results were reduced to the value for 0.05N acid assuming that the velocity coefficients were proportional to the concentration of catalyst.

TABLE I

Dimethyl azelate and ethyl alcohol

Volume of dilatometer 42.5 c.c. ; capillary 0.00175 c.c./cm.
Volume of dilatometer for blank 42.0 c.c. ; capillary 0.00140 c.c./cm.
Conc. of HCl 0.1046N.—Ester 1.3 g. in 50 c.c. or 138 : 1 mols.

Time mins.	Change in blank, mm.	Change in reaction capillary, mm.	Corrected change	$K \times 10^5$
37	−0.2	3.2	3.05	246
75.5	−1.0	6.5	5.7	237
100	−1.4	8.7	7.6	246
150	−0.3	10.9	10.65	244
209	−0.4	14.5	14.2	251
274	−1.1	17.8	16.9	242
330	−0.7	19.7	19.15	242
458	−5.2	27.5	23.35	242
1200	−3.3	35.2	32.55	...
1430	0.8	32.9	33.55	...
1470	2.0	32.9	34.5	...
1780	0.9	34.1	34.8	...
2500	−2.7	37.0	34.85	...
∞	...	...	34.85	...
...	...	...	...	mean 244

$K \times 10^{-5}$ for 0.50N. acid = 117

In Table II further results are given in an abbreviated form. N denotes the normality of the acid, C the weight or volume of ester in 50 c.c., t the time of reaction in minutes, x the total movement of the meniscus in the dilatometer tube (corrected) in mm., k, $K \times 10^5$ and k' $K \times 10^5$ for 0.05 N acid,

TABLE II

Dimethyl oxalate, ethyl alcohol.

$N=0.0267$ $C=2.303$g.			$N=0.1004$ $C=2.685$g.		
t	x	k	t	x	k
81	10.7	141	65	27.5	504
157	20.2	144	84	34.1	504
232	28.5	145	109	42.4	506
298	35.0	145	135	49.5	515
356	40.1	145	183	60.6	522
464	49.0	146	263	73.6	520
576	55.8	143	327	81.0	529
1281	84.3	146	384	85.8	534
∞	99.6	...	∞	98.5	...
$k_m=144$ $k'=270$			$k_m=518$ $k'=258$		

Diethyl oxalate, methyl alcohol.

$N=0.0264$ $C=2.0$c.c.			$N=0.0525$ $C=2.5$c.c.		
t	x	k	t	x	k
34	10.7	421	16	11.0	849
58	17.2	416	27	17.1	815
98	26.7	414	44	26.3	821
138	34.6	410	63	34.9	819
207	44.5	403	108	50.3	805
298	55.2	391	145	59.3	796
357	60.4	393	188	67.2	796
∞	80.1	...	214	70.6	788
...	...	...	∞	86.6	...
$k_m=407$ $k'=771$			$k_m=800$ $k'=762$		

Dimethyl malonate, ethyl alcohol.

$N=0.0977$ $C=2.05$c.c.			$N=0.1876$ $C=2.05$c.c.		
t	x	k	t	x	k
142	13.6	78.7	60	11.2	152
222	21.2	81.2	131	23.9	158
285	26.7	81.7	180	31.1	154
442	39.5	83.0	245	40.8	157
555	47.8	84.0	307	48.8	156
614	51.4	83.0	399	51.5	157
1276	83.4	82.0	∞	128.1	...
1435	89.3	81.0	...	...	...
1649	95.1	81.7	...	...	...
1990	102.4	80.0	...	...	...
∞	128.5	...	...	...	...
$k_m=81.6$ $k'=41.9$			$k_m=156$ $k'=41.4$		

Diethyl malonate, methyl alcohol.

$N=0.0522$ $C=2.6$ c.c.			$N=0.0523$ $C=2.6$c.c.		
t	x	k	t	x	k
30.5	6.7	151	29	6.4	155
54.5	11.8	151	76	15.9	151
90	18.4	146	133	26.7	151
150	29.0	144	214	41.0	153
221	40.8	144	291	52.2	152
306	53.1	143	328	57.0	150
406	66.1	143	489	76.0	149
672	92.2	142	1214	123.5	151
1308	128.5	150	∞	146.8	...
1406	131.2	150	...	...	...
∞	149.8	...	...	...	...
$k_m=146$ $k'=140$			$k_m=151$ $k'=144$		

Dimethyl succinate, ethyl alcohol

$N=0.0494$ $C=2.3$ c.c.			$N=0.1052$ $C=3.0$ c.c.		
t	x	k	t	x	k
89	6.5	58.2	59	11.7	124
156	11.7	61.4	109	21.0	124
223	16.2	60.5	155	28.8	123
280	20.2	61.0	205	36.0	120
408	28.3	60.5	266	45.4	120
496	33.3	60.7	377	56.5	123
1159	63.8	58.9	457	70.0	119
1409	72.9	59.1	632	86.7	116
1694	81.5	58.9	1253	127.5	116
1869	85.9	58.2	1713	145.7	121
2664	102.3	59.1	∞	166.4	...
2967	107.7	60.5	...	...	...
∞	129.0	...	...	...	...

$k_m=59.9$ $k'=60.6$ $k_m=121$ $k'=57.5$

Diethyl succinate, methyl alcohol

$N=0.0456$ $C=2.0$ c.c.			$N=0.4046$ $C=2.0$ c.c.		
t	x	k	t	x	k
46	7.4	204	23	6.9	421
108	15.5	192	44	12.7	421
136	19.0	192	86	22.5	414
210	27.7	195	157	36.1	419
256	32.4	194	278	51.5	416
316	37.7	193	379	60.1	426
403	64.6	193	∞	75.0	...
579	56.1	192	...	...	...
∞	82.6	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...

$k_m=193$ $k'=212$ $k_m=419$ $k'=200$

Dimethyl adipate, ethyl alcohol

$N=0.0523$ $C=2.9$ c.c.			$N=0.1001$ $C=3.05$ c.c.		
t	x	k	t	x	k
61	8.8	131	68	15.2	219
110	14.9	126	110	23.8	222
175	22.8	127	157	32.4	222
271	32.8	124	208	40.9	224
350	40.2	123	274	50.4	224
506	53.1	123	389	64.2	225
640	63.1	125	471	72.2	227
1373	94.7	127	622	82.9	225
∞	114.7	...	∞	110.0	...
...	...	...	...	...	...

$k_m=125$ $k'=120$ $k_m=224$ $k'=112$

Diethyl adipate, methyl alcohol

$N=0.0532$ $C=3.0$ c.c.			$N=0.1122$ $C=3.0$ c.c.		
t	x	k	t	x	k
20	9.2	460	15	14.5	1060
43	19.0	465	25	22.8	1050
75	31.2	472	40	33.2	1025
97	38.1	465	79	54.7	1020
135	50.1	481	95	61.7	1030
188	61.6	472	121	70.3	1025
240	70.8	469	149	77.6	1000
317	81.2	469	186	84.6	1040
450	92.6	479	∞	98.8	...
∞	104.8	...	...	...	...

$k_m=470$ $k'=442$ $k_m=1030$ $k'=459$

Dimethyl suberate, ethyl alcohol

$N=0.0578$ $C=2.9$ c.c. $N=0.1046$ $C=2.9$ c.c.

t	x	k	t	x	k
51	5.5	142	73	14.4	237
102	10.7	143	120	22.6	237
182	17.6	139	172	30.4	237
247	22.6	137	214	36.2	237
347	29.8	137	271	42.7	235
407	33.2	135	325	48.5	235
561	43.5	144	376	53.1	235
1298	66.1	141	503	63.2	237
1458	68.9	142	683	73.0	239
∞	78.6	...	∞	90.8	...

$k_m=140$ $k'=121$ $k_m=237$ $k'=113$.

Dimethyl sebacate, ethyl alcohol

$N=0.0523$ $C=2.8$ c.c. $N=0.1046$ $C=3.0$ c.c.

t	x	k		x	k
60	4.5	127	84	12.1	232
116	8.3	126	127	17.7	237
227	15.2	125	184	75.2	250
403	24.4	126	253	31.9	250
1200	47.6	126	343	39.3	251
1561	53.0	129	480	46.6	239
∞	61.1	...	∞	68.1	...
...	...	...	...	...	...
...	...	...	...	...	...
...	...	...	...	...	...

$k_m=126$ $k'=121$ $k_m=243$ $k'=116$.

Diethyl tartrate, methyl alcohol

$N=0.059$ $C=2.323$g. $N=0.1095$ $C=2.24$g.

t	x	k	t	x	k
41	3.5	81.9	34	5.6	185
97	8.2	82.5	54	8.4	180
158	12.9	81.6	90	13.5	178
240	18.8	80.9	108	15.8	177
308	23.1	79.1	147	20.8	177
358	26.0	77.9	200	27.3	179
454	32.3	79.3	246	32.5	180
1311	68.8	79.1	517	55.9	185
1435	72.3	79.1	∞	90.7	...
1763	79.6	77.9	...	...	...
∞	106.5	...	...	...	...

$k_m=80.0$ $k'=76.5$ $k_m=180$ $k'=82.3$.

Diethyl malate, methyl alcohol

$N=0.0423$ $C=2.6$ c.c. $N=0.106$ $C=2.55$ c.c.

t	x	x calc.	t	x	x calc.
33	9.0	9.0	31	13.4	13.3
65	16.6	16.6	58	21.9	21.9
107	25.2	25.2	82	28.1	28.0
167	35.6	35.4	113	34.2	34.2
201	40.3	40.3	168	41.7	41.8
250	46.3	46.3	233	47.5	47.7
289	50.5	50.5	307	52.0	52.3
511	66.4	66.4	456	58.4	58.2
1234	84.6	86.5	668	64.0	63.4
∞	102.0	...	∞	74.6	...
...	...	...	...	...	...

$k_1=469$ $k'_1=554$ $k_1=1115$ $k'_1=545$

$k_2=98.5$ $k'_2=116$ $k_2=184$ $k'_2=87$

Examination of the figures in Table II reveals the fact that with dimethyl oxalate there is a slight tendency for the values of k to increase as the reaction proceeds, while in the case of diethyl oxalate the reverse is to be seen. This would imply that the OMe group in EtOOC.COOMe is replaced more readily than it is in MeOOC.COOMe, and that OEt in EtOOC. COOEt is replaced more readily than in EtOOC.COOMe; in other words, the methyl group causes a slight retardation. The experimental results are not sufficiently accurate to allow the two velocity coefficients to be calculated. A similar effect is not seen in the case of the esters of other symmetrical acids, doubtless owing to the wider separation of the reacting groups. In all these cases the reaction appears to be a unimolecular one within the limit of experimental error indicating that the velocity of replacement of both groups is the same.

With diethyl malate the results are of a different order. The value of k calculated according to formula (3) fell from 279 to 143 with the more dilute catalyst and from 635 to 292 with the more concentrated. On applying equation (4) with the values of K_1 and K_2 shown in the table, a very satisfactory agreement between observed and calculated values was found, indicating that the velocity coefficient for one group was about five times that for the other. The values for K_2 are not in very good agreement possibly owing to the fact that as the reaction approaches completion the simple equation no longer holds, and also because a small experimental error towards the end of the experiment has a large effect on the value of K_2.

The values of K (the velocity coefficient calculated for 0.05N acid) for all the esters with the single exception of diethyl adipate are smaller for the more concentrated catalyst, indicating that the more dilute acid is relatively more active, although the differences are slight and in many cases not greater than the experimental error.

The examples given show that the concentration of ester decreases in an exponential manner towards the equilibrium point as a limit. This is necessarily an approximation since the reaction is in reality more complex, particularly towards the end when the reverse reaction becomes appreciable. In most cases it is possible to calculate the equilibrium constant with sufficient accuracy and from this to deduce the true velocity coefficients. For example in the case of the esters of succinic acid k' is 60.6 for the methyl ester and 212 for the ethyl ester at catalyst concentrations very near to 0.05N. Hence the equilibrium constant is approximately 212/60.6 or 3.50. The initial ratio of molecules of alcohol to ester is 46 :1 in the first reaction and 99 :1 in the second.

Hence if x and y are the molecular concentrations of original ester at equilibrium, the initial concentration being 1

$$x\,(45+x)/(1-x)^2=(1-y)^2/y\,(98+y)=3{\cdot}5$$

whence $x=0.067$ and $y=0.003$. In other words 6.7 per cent of ester remains unchanged at equilibrium in the first case, while in the second the conversion is over 99 per cent. The total volume change for the methyl ester would thus be 138 if the reaction went to completion and a more accurate value for k is obtained by using this figure instead of 126 and recalculating the values for the initial stages only. It may be shown that when v is small, $\ln(1\text{-}v/V)$ is inversely proportional to V so that if V increases by $100x$ per cent in formula (4), k diminishes in the same proportion in the initial stages. Since in most of the reactions studied k was constant within the limit of experimental error, a sufficient approximation to the correct value can be obtained by diminishing the mean k_m by $100x$ per cent and this has been done in the following table. The actual values of x varied from 0.051 to 0.072 for all the methyl esters except methyl azelate for which it was only 0.028 owing to the small quantity used. For the ethyl esters the value was in all cases 0.004 or less and no correction has been applied. For the three esters in which only one reaction was studied, the equilibrium constant was assumed to be 4.0

Table III.

Corrected velocity coefficients for 0.05 N acid

Acid		Methyl ester	Ethyl ester	Equilibrium constant	Esterification (Kailan)
Oxalic	...	248	762	3·25	414
Malonic	...	39·5	142	3.6	142
Succinic	...	56.3	212	3·75	207
Adipic	...	111	442	4.0	336
Suberic	...	113	...	...	...
Azelaic	...	114	...	...	...
Sebacic	...	114	...	...	...
Tartaric	...	...	76.5	...	68
Malic (*a*)	...	...	554	...	284
(*b*)	...	...	116	...	...

It will thus be seen that the velocity coefficients are greatest in the case of oxalic acid and least for malonic acid; as the number of carbon atoms increases the coefficients also increase until a constant value is reached. These results are analogous to those of Kailan (*loc. cit.*) for the velocity of esterification, which are also given in the table after multiplying by a factor to equalise the values for malonic acid. This author did not extend his measurements to the higher acids of the series. He states that the values for oxalic and malonic acids were not very reliable because decreasing results were obtained as the reaction proceeded, but the large difference between the coefficients for alcoholysis and esterification in the case of the former acid is somewhat remarkable and can scarcely be attributed to experimental error or the action of water.

Kolhatkar's figures for the monobasic acids show that methyl acetate reacts with ethyl alcohol nearly twice as rapidly as methyl oxalate under similar conditions ($k=430$). There is however, no minimum at the second member in this series, k for methyl propionate and butyrate being 245 and 137 respectively. The last figure approaches that for the higher dibasic acids (114) and it seems probable that if measurements were extended to long-chain monobasic acids, the figures for the two series would coincide. It appears therefore that the replacement of CH_3 in the monobasic acids by COOMe produces a large retardation for the second and third members and little or no effect when the number of carbon atoms is increased. These results bear a close resemblance to the effect of introducing an *iso*propyl group into the normal fatty acid molecule in the case of esterification as shown by Bhide and Sudborough (*J. Indian Inst. Sci.*, 1925, **8**, 91) their results being reproduced in Table IV together with those of Kolhatkar and some of the present series.

Table IV.

Alcoholysis Ester	k	*Esterification* Acid	k
$CH_3.COOMe$	430	$CH_3.COOH$	796
$MeOOC.COOMe$	248	$(CH_3)_2CH.COOH$	156
$CH_3.CH_2.COOMe$	245	$CH_3.CH_2.COOH$	550
$MeOOC.CH_2.COOMe$	40	$(CH_3)_2CH.CH_2COOH$	52
$CH_3(CH_2)_2\ COOMe$	137	$CH_3(CH_2)_2.COOH$	270
$MeOOC.(CH_2)_2.COOMe$	56	$(CH_3)_2CH.(CH_2)_2.COOH$	247
$CH_3.(CH_2)_4.COOMe$	?	$CH_3.(CH_2)_3.COOH$	268
$MeOOC.(CH_2)_4.COOMe$	111	$(CH_3)_2.CH.(CH_2)_3.COOH$	267

In both cases the maximum retardation occurs when the replacement is made on the carbon atom in the β-position.

The above authors have also measured the esterification velocity coefficients for adipic, pimelic and suberic acids and have recorded a slight decrease, the values in arbitrary units being 307, 282 and 264 respectively. These results appear somewhat abnormal.

Kailan did not calculate the two velocity coefficients for malic acid, but his figures approximate to those obtained for alcoholysis. The substitution of one atom of hydrogen in succinic acid by OH thus accelerates the velocity of reaction of one COOH or COOMe-group and retards that of the other. Kailan concluded from his results with mandelic and phenylacetic acids (*Sitzungsber. Akad. Wiss. Wien,* 1907, **116**, IIb, 1094) that an OH group in the α-position slightly retards the rate of reaction and consequently that the COOH-group next to the CHOH-group in malic acid esterifies less rapidly. Drushel (*Amer. J. Sc.,* 1915, IV, **39**, 113), however, from a study of the hydroxypropionic esters has shown that in the case of hydrolysis, the substitution of an OH-group in the α-position accelerates the reaction and has a marked retarding effect in the β-position, the coefficients for ethyl propionate and α-and β-hydroxypropionic esters being 71.2, 76.1 and 16.3 respectively.

Retardation by a β-substituent is in accordance with the results given in Table IV and it seems probable that Kailan's assumption is incorrect and that the COOH-group adjacent to the CH_2-group in malic acid is the less reactive. This retarding effect would also explain to some extent the very low value for tartaric acid.

The equilibrium constants of the dibasic esters increase from 3.25 to 4.0 for the members of the series from oxalic to adipic acid, a variation somewhat greater than that found by Kolhatkar for the ethyl and methyl esters of acetic, propionic and *n*-butyric acid for which the values were 3.5, 3.8 and 3.7 respectively.

We wish to take this opportunity of expressing our thanks to Mr. S. K. Kulkarni Jatkar for much assistance during the course of our experiments.

SUMMARY

1. The velocity of alcoholysis of methyl and ethyl esters of oxalic, malonic, succinic, adipic, suberic, azelaic, sebacic, tartaric and malic acids with ethyl and methyl alcohol in presence of hydrochloric acid as a catalyst has been studied at 30° by a dilatometric method.

2. In the case of the symmetrical acids the reaction proceeds according to the rule for a unimolecular reaction, indicating that the replacement of OMe by OEt at one end of the molecule does not appreciably affect the rate of reaction of the other COOMe-group. Oxalic acid is an exception but the departure from the rule is so small that the two velocity coefficients cannot be calculated with accuracy.

3. The two ester groups in malic acid react at different rates, the one adjacent to the CH_2-group being apparently the less reactive. The velocity observed agrees with that calculated on the hypothesis that two unimolecular reactions take place simultaneously at different rates.

4. In the series of unsubstituted acids, oxalic acid has the largest velocity coefficient and malonic acid the smallest. The values for adipic acid and the succeeding members are all equal.

5. The reaction velocity is very nearly proportional to the concentration of catalyst in the range studied.

6. The equilibrium constant increases slightly from oxalic to adipic acid.

7. Alcoholysis of these acids bears a considerable resemblance to the esterification as measured by Kailan.

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DILATOMETRIC STUDIES IN ENZYME ACTION. PART III.

Contraction constants of enzyme-substrate reactions.

By H. B. Sreerangachar and M. Sreenivasaya.

GLUCOSIDES.

The present communication deals with the hydrolysis of four well-known glucosides, amygdalin, arbutin, aesculin and salicin by emulsin, the reactions being conducted in the dilatometer described in an earlier paper (*J. Indian Inst. Sci.*, 1932, **15A**, 17), which gives the experimental procedure in detail. In the present study, the fall in the dilatometric column was measured at intervals; the degree of hydrolysis was also determined by an independent analysis of the reaction mixture, 5 c.c. aliquots of which, were drawn from a control flask maintained for the purpose. The amount of sugar thus determined gives the amount of glucoside hydrolysed. The contraction constant per gram molecule of the glucoside can therefore be calculated from the quantity hydrolysed and the corresponding dilatometric depression observed.

EXPERIMENTAL.

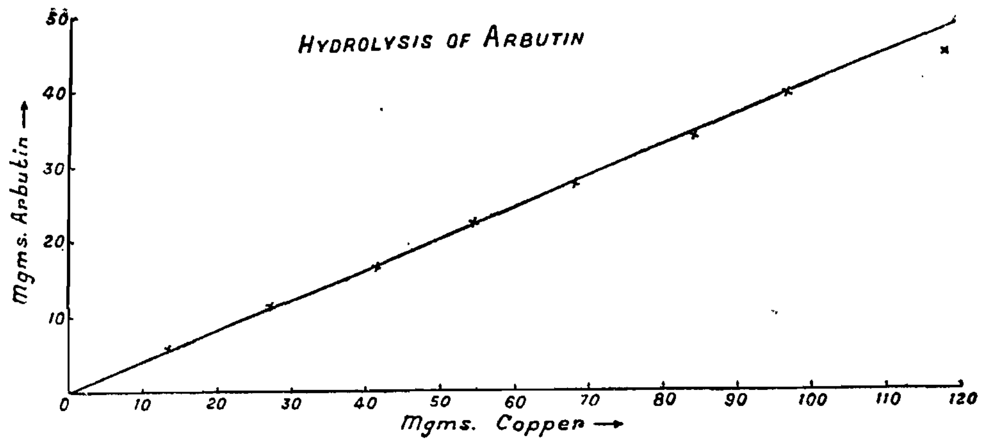
Mixtures containing 50 c.c. of about 1 per cent. solution of the glucoside dissolved in Sörensen's phosphate buffer (P 5.2) and 5 c.c. of 1 per cent. solution of almond emulsin (B.D.H.) were usually employed for the experiments. Aesculin and arbutin were preparations of Theodor Schuchardt, amygdalin, Kahlbaum's, and salicin, B.D.H.

Sugar formed in the hydrolysates was estimated by Bertrand's method. In the case of arbutin, however, the estimation had to be carried out immediately, since the hydroquinone accompanying the sugar in the hydrolysate became rapidly oxidised to quinone particularly in alkaline solutions. In calculating the amount of arbutin from the experimental copper value, the combined reducing action of sugar and hydroquinone have to be considered.

Copper values for mixtures of the two reducing constituents in the proportion in which they occur in arbutin were determined, and the values given in Table I are represented in the graph, from which, given the copper value of arbutin hydrolysate, the amount of glucoside hydrolysed can be directly read.

TABLE I

	1	2	3	4	5	6	7	8
Mixture, c.c. ...	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Copper reduction, mg. ...	13.8	27.1	41.4	54.7	68.5	84.8	97.1	118.3
Arbutin, mg. ...	5.6	11.2	16.8	22.4	27.5	33.5	39.1	44.7



Reduction values should be determined immediately after pipetting the arbutin hydrolysate or the artificially prepared sugar-hydroquinone mixture into the alkaline copper solution. Atmospheric oxygen quickly oxidises the hydroquinone in alkaline media as revealed by the following table, in which the results of back-titrating unused ferric iron according to the Bertrand method at different intervals, are given.

TABLE II

Time of estimation.	$KMnO_4$ (*N*/10) required in c.c.
Immediate.	5·95
After 1 hour.	4·70
After aeration for 1 hour.	2.85

TABLE III

Emulsin—amygdalin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Amygdalin hydrolysed in mg.	*Dilatometer readings in cms.*		Dilatometric depression
			Exptl.	Control	
23	28.6	165.8	2.8	16.7	0.0
46	47·0	330·3	2.8	16.7	0.0
78	51.6	363.8	2.8	16.7	0.0
135	59·8	424·5	2.8	16.8	0.0

TABLE IV

Emulsin—arbutin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Arbutin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression
			Exptl.	Control	
100	15.3	70.4	4.3	12.2	0.0
194	25.2	116.1	4.3	12.2	0.0
342	39.2	176.0	4.3	12.2	0.0
610	62.3	279.4	4.3	12.2	0.0
1230	83.8	375.1	4.4	12.3	0.0

TABLE V

Emulsin—Salicin.

Time in mins.	Copper equivalent of 5 c.c. of hydrolysate in mg.	Salicin hydrolysed in mg.	*Dilatometer readings* in cms.		Dilatometric depression cms.	Contraction constants
			Exptl.	Control.		
...	...	...	4.2	5.4	...	...
28	9.8	89.8	5.2	5.4	1.29	4.12
80	17.8	172.4	7.1	5.4	2.46	4.08
236	14.4	139.2	8.6	5.4	2.00	4.11

Since a preliminary study of the emulsin-aesculin system revealed no volume-change while the hydrolysis proceeded to the equilibrium point, no contraction constant can be obtained for this system.

DISCUSSION.

Of the four glucosides, the hydrolyses of which have been dilatometrically investigated, salicin is the only one which gives a depression and has a contraction constant. The absence of any volume-change during the enzymatic splitting of the other three glucosides is noteworthy.

To eliminate the possibility of counter volume-changes which might accompany the solution of the reaction products in petroleum, experiments were conducted with the old type of instrument which eliminates the use of the solvent. Neither this gave any depression, showing that the factor of solubility in petroleum does not affect the result. Attention should be drawn to the fact that the volume-change measured in the dilatometer is the net result of a series of physical and chemical changes accompanying the hydrolysis, such as the solubility of the hydrolytic products in the reaction media, the disappearance of water during hydrolysis, and the configurational readjustment of the new molecules resulting from hydrolysis. It is difficult at this stage to apportion the change to each of the above factors, but an attempt will be made on a future occasion when a greater variety and number of substances would have been investigated.

SUMMARY.

The hydrolysis of four glucosides, aesculin, amygdalin, arbutin and salicin by emulsin has been investigated in the dilatometer.

Emulsin-salicin gives a contraction constant of 4.1 while other systems do not reveal any volume-change during hydrolysis.

POLYSACCHARIDES.

A dilatometric study of the enzymatic hydrolysis of the colloidal polysaccharides starch and glycogen is described in the present communication. As previously mentioned (*J. Indian Inst. Sci.*, 1932, **15A**, 17) the contraction constant is expressed in cubic centimetres per 100 grams of the colloidal substrate. The preparation of solutions of these collodial substrates under standard and reproducible conditions is of great importance in these experiments. For example, the hydration of a given starch is regulated by hydrogen ion, the presence or absence of salts, and temperature. Since this hydration itself is accompanied by certain volume changes, the starch solutions were kept in the thermostat for 19 hours, so that the hydration equilibrium was attained before mixing the substrate and the enzyme.

EXPERIMENTAL.

Lintner's soluble starch (B.D.H.) was used throughout the experiments : glycogen was a Pfansteil's c.p. sample. They were subjected to the action of four different diastases, widely varying in origin. Pancreatin and malt diastase were Merck's preparations ; ptyalin was prepared in the laboratory, and Taka-diastase was a product of Parke Davis.

Except in the case of glycogen, all hydrolyses were conducted at four different concentrations of the substrate. Completion of the reaction was determined by the dilatometric column reaching a steady

value, usually in the course of 4 to 5 hours. The degree of hydrolysis was independently determined by estimating the reducing value of the hydrolysate by Bertrand's method. For both series of studies, the respective optimal reactions were maintained with Sörensen's phosphate buffer :—Pancreatin, 7.0 ; ptyalin, 6.6 ; malt and Taka-diastase, 5.3.

TABLE I

Starch

Substrate concentration per cent.		0.25	0.50	1.0	2.0	Average constant
Pancreatin	Observed depression in cm.	0.65	1.45	2.7	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	8.9	17.2	34.3	73.8	
	Contraction constant	0.64	0.71	0.66	0.71	0.68
Ptyalin	Observed depression in cm.	0.7	1.6	2.65	5.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	...	...	41.34	80.14	
	Contraction constant	0.69	0.65	0.65	0.71	0.67
Diastase	Observed depression in cm.	0.8	1.60	3.2	6.5	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	10.8	...	38.2	85.2	
	Contraction constant	0.79	0.79	0.79	0.79	0.79
Taka-diastase	Observed depression in cm.	1.6	3.15	6.25	13.0	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	16.5	40.55	67.4	136.1	
	Contraction constant	1.57	1.55	1.55	1.59	1.57

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TABLE II

Glycogen

Substrate concentration per cent.		0.25	0.5	Average constant
Pancreatin	Observed depression in cm.	0.45	0.95	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.1	11.8	
	Contraction constant	0.44	0.47	0.45
Ptyalin	Observed depression in cm.	0.85	1.8	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	6.6	13.8	
	Contraction constant	0.83	0.88	0.85
Diastase	Observed depression in cm.	0.55	1.1	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	7.7	15.3	
	Contraction constant	0.54	0.54	0.54
Taka-diastase	Observed depression in cm.	1.5	2.9	
	Reducing value in mg. of copper for 5 c.c. hydrolysate	23.0	42.4	
	Contraction constant	1.47	1.43	1.45

DISCUSSION.

Tables I and II reveal that highest depressions are obtained with Taka-diastase at all concentrations of the substrates tested. The highest degree of hydrolysis is brought about by Taka-diastase as indicated by the corresponding copper values also. A strict proportionality (within reasonable limits of experimental error) is maintained between the various concentrations not only with respect to dilatometric depressions but also with regard to the copper values of the hydrolysate. The contraction constants of each system obtained at various concentrations of the substrate show good agreement and therefore these constants can be used for the estimation of starch or glycogen. Taka-diastase, giving the highest constant, should prove most useful in the analytical dilatometry of starch or glycogen.

The results show that the degree of hydrolysis of polysaccharides can be followed in the dilatometer, although it may be difficult to follow the disruption of the molecule ; there is no doubt that each of these diastases act differently on starch. We are dealing here with a complex substrate consisting of amylopectin and amylose, the former according to Pringsheim being closely related to glycogen. The enzyme preparations represent a mixture of several saccharifiers and one liquefying component. Therefore a detailed study of this system by effecting a separation of the components of the substrate and enzyme systems is being made.

SUMMARY.

1. Starch and glycogen in concentrations of 0.25, 0.5, 1.0 and 2 per cent. have been separately subjected to the action of four diastases differing widely in origin, pancreatin, ptyalin, malt and Taka-diastase, in the dilatometer.

2. Contraction constants for each of these eight systems have been determined and the possibility of their being employed for the dilatometric estimation of starch and glycogen with particular reference to Taka-diastase, has been indicated.

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[Accepted, 29-3-33.]

PHYSIOLOGICAL PRODUCTS OF THE LAC INSECT. PART I.

A preliminary investigation.

By N. K. Ranga Rao and M. Sreenivasaya.

Although modern trade and industry value lac primarily for its resinous incrustation, yet from the viewpoint of ancient Hindu medicine, the insect residues, which consist of nitrogenous and fatty bodies, have a therapeutic interest. Aqueous decoctions of the insect are administered in lung diseases. Germicidal, febrifugal and astringent properties are also attributed to lac which, finely powered and mixed with honey, is administered as a specific for haemostosis. It forms the essential ingredient of a medicated oil *Lakshadi Thaila,* which is reputed to reduce chronic fevers, cure rheumatic pain and help the growth of foetus during pregnancy.

Ayurvedic literature or practice does not identify the component of stick lac to which the medicinal property is due. It is not known whether the brood lac with its living population of mother insects, or "phunky" lac containing the debris of insect skins, is to be employed for the above-mentioned preparations. Clinical experience points to the conclusion that the most efficacious and potent preparations are obtained from fresh brood lac before swarming ; it is also suggested that wild lac, *Lakshadia communis,* which is usually found on *Ficus* trees, possesses a higher medicinal value. When aqueous decoctions are administered, the dissolved body fluids of the insect are involved, but the medicated oil should incorporate exclusively the wax and the insect fat, both of which probably constitute active ingredients.

A detailed study of the nitrogenous and fatty constituents of the insect has been undertaken in view of its importance in Hindu medicine. This investigation has an additional interest of economic value since in the washing of stick lac in North Indian factories, these valuable constituents run to waste. The wash-liquors should certainly yield products of economic value.

EXPERIMENTAL.

The brood lac employed in these studies was obtained from one of the plantations at Devarabetta, North Salem, three weeks before

the commencement of larval swarming. The insect mothers inhabiting the incrustation had therefore very nearly reached their maximum maturity, since this stage represented the peak on the growth curve. The incrustation was washed in a rapid stream of water to dissolve the honey-dew deposited on the surface. After wiping with clean cloth, the incrustation which was removed from the green twigs and carefully freed from plant-debris, provided the starting material for all subsequent investigations.

The incrustation (20 lbs.) was crushed in a porcelain end runner and extracted with 10 litres of 0.9 per cent. sodium chloride solution. The decanted liquid was passed through a cloth filter and the residue pressed in a hydraulic press at 2 tons to the square inch to recover as much of the extract as possible. On centrifuging at 3,000 R.P.M., the combined filtrates separated into three distinct layers :— (1) the uppermost layer consisting of scum which could be easily skimmed, (2) the middle layer, a thick, fairly clear scarlet fluid and (3) the residues comprising the chitinous debris of the insect and the finer particles of lac incrustation escaping the cloth filter.

The butter-like insect fat was easily separated, the middle layer carefully decanted and the residues kept separately. The fat was desiccated *in vacuo* over calcium chloride and subsequently purified by extraction with carbon tetrachloride forming a pale yellow solid.

The middle portion was filtered with considerable difficulty through fluted filters in the cold room at 0°, and the filtrate (about 10 litres) divided into three portions. The first portion was saturated with magnesium sulphate, and the second treated with ammonium sulphate to half saturation. In these cases small quantities of precipitate were obtained having nitrogen values of 7.6 and 7.5 per cent. respectively. On heating to 90°, the third portion did not yield a precipitate and was therefore treated with a few drops of acetic acid, the precipitate thus obtained containing 8.7 per cent. total nitrogen. The following is a schematic representation of the treatments.

Incrustation (20 lbs.)

Treated with 10 litres of sodium chloride (0·9 per cent.) solution

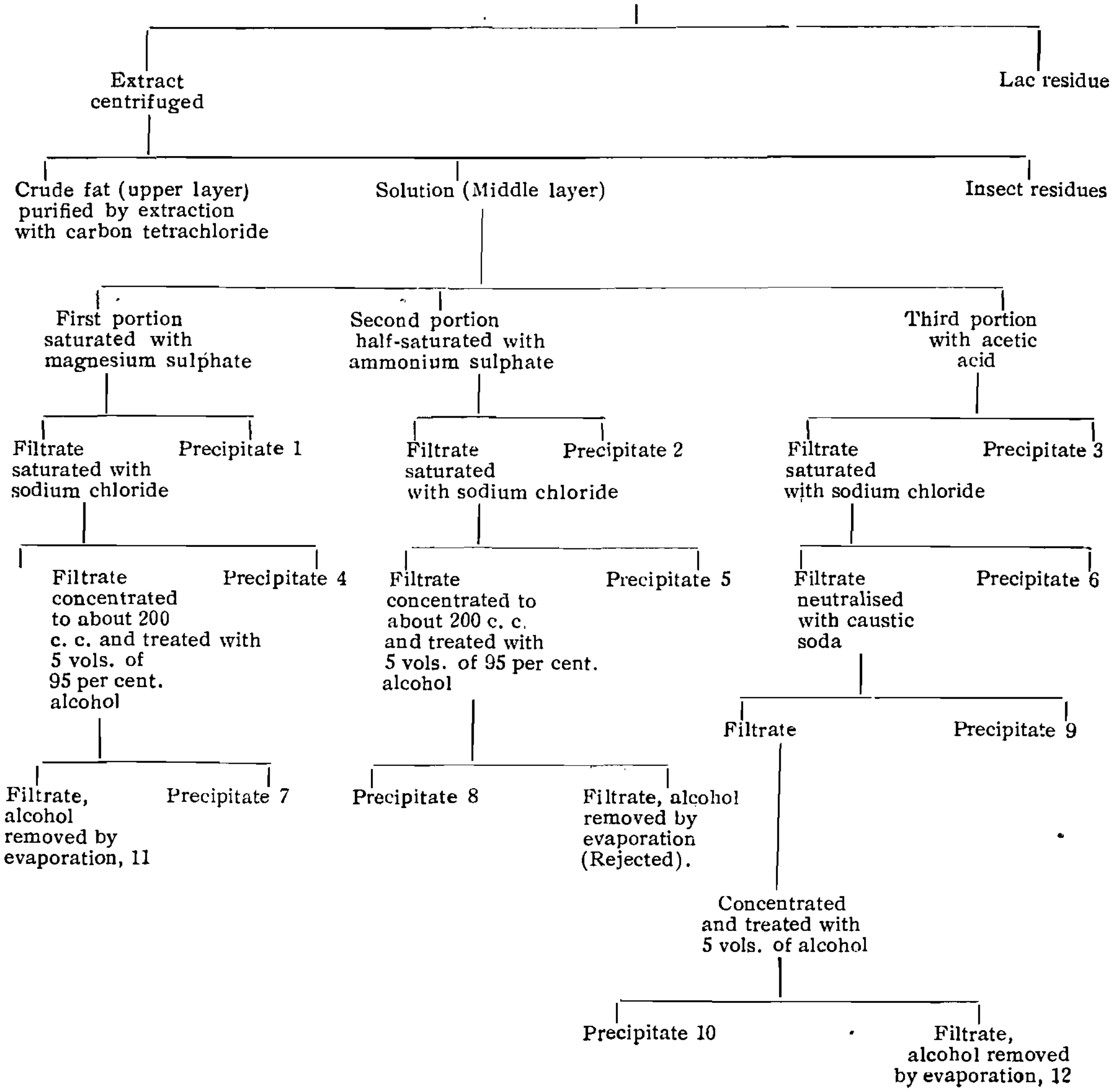

Table I gives the general appearance and characteristic properties of the above twelve fractions.

TABLE I

Fraction No.	Wt. of product gms.	Appearance	Nitrogen value per cent.	Other properties
1	1.0	Brick red powder	7.6	Amorphous
2	1.2	do.	7.5	do.
3	0.8	Dark red	8.7	do.
4	3.0	do.	2.72	Amorphous, answers biuret and α-naphthol tests
5	...	Light pink	...	Crystalline, ammonium sulphate
6	4.0	Dark red	3.34	Answers biuret test
7	6.0	Flesh colour	...	Highly hygroscopic, does not reduce Fehlings' solution answers α-naphthol test
8	...	Light pink	...	Crystalline, ammonium sulphate
9	5.0	Bright pink	0.00	Contains no nitrogen
10	7.0	Light brown	0.70	Soluble in water, answers α-naphthol test
11 & 12	250 c.c.	Scarlet liquid	11.0 (on the ash-free total solids)	

It will be seen from Table I that the three albuminous precipitates 1, 2, and 3 obtained by the three different methods contain about the same percentage of total nitrogen. The protein exists in combination with the strongly acidic laccaic acid which is responsible for the deep red colour of the preparations. The substance precipitated by acetic acid has a higher nitrogen value of 8.7. When purified by dissolving it in dilute caustic potash and reprecipitating with acetic acid the product

gave a nitrogen value of 12.1 per cent. Further purification did not increase the nitrogen content.

The Hausmann numbers of the first, second and third fractions as also the final solution (11 and 12) have been determined with a view to finding out the proximate proportions of the basic and non-basic forms of nitrogen.

TABLE II

Fractions	Percentage of total nitrogen				
	1	2	3	4	11 and 12
Humin (insoluble)	2.08	2.02	1.94	5.24	5.65
,, (soluble)			0.23	18.32	3.38
Amide	13.8	13.29	14.36	10.37	4.52
Basic	25.0	24.15	25.89	20.46	45.81
Non-basic ...	58.5	58.83	58.25	42.54	40.67
Total ...	99.38	98.29	100.64	96.93	100.03

Table II shows that the first three fractions have approximately the same composition, the fourth fraction on acid hydrolysis yields a high proportion of humin nitrogen due to the presence of carbohydrates as indicated by the α-naphthol test. The solution is characterised by a high percentage of the basic nitrogen.

The most interesting among the nitrogenous bodies which merited detailed investigation appeared to be the first three fractions representing the serum albumins of the lac insect and the residual serum (11 and 12) consisting of the simpler polypeptides. The third fraction, after purification as previously indicated, and the residual serum were subjected to Van Slyke analysis. In the solution the basic fraction was further investigated by Kossel's silver baryta method.

TABLE III

Forms of Nitrogen	Percentage of Total Nitrogen		
	fractions		Portamine-like substance from *Sardinia coerulea* (1)
	3	11 and 12	
Humin	...	...	2.83
Acid-insoluble ...	1.94	5.65	...
Acid-soluble ...	0.23	3.38	...
Amide	14.36	4.52	0.86
Basic	25.89	46.41	...
Arginine	8.09	21.02 20.31*	27.83
Histidine	5.99	23.70 23.28*	23.02
Lysine	11.81	1.69	5.48
Cystine	Nil	Nil	0.60
Non-basic	58.25	40.67	40.22
Total	100.67	100.63	100.84

* Kossel's method.

DISCUSSION.

Table III gives the results of analysis. The serum albumin has high proportions of basic and amide-nitrogen and in general corresponds in composition to the vegetable globulins. The serum-nitrogen not precipitated by saturation with any of the salts, consists of a series of lower peptones and polypeptides. The fact that this fraction contains 11.0 per cent. of nitrogen on the total solids, indicates that the nitrogenous bodies in solution are moderately pure. About 9 per cent. of the total nitrogen exists in the amino-form, which increases to 43 per cent. on acid hydrolysis. This small (less than five times) increase in amino-nitrogen shows that the nitrogenous materials in solution are not complex in structure and consist of a series of the simpler polypeptides. The free amino-nitrogen of gelatin is only 0.1 per cent. of

its total nitrogen ; on hydrolysis, however, the amino-nitrogen increases to about 60 per cent., i.e., 600 times.

The high arginine- and histidine-content of the serum is noteworthy, and ranks this complex with the protamine-like substance isolated by Dunn (*J. Biol. Chem.*, 1926, **70**, 697) from the testicles of *Sardinia coerulea*. Analytical data for this protamine are presented for comparison in Table III, showing close agreement between Van Slyke and Kossel methods with regard to the arginine and histidine-content. Fractionation of the serum with a view to isolating individual polypeptides is now in progress.

The fat.—The fat isolated during the process of centrifuging and purified by extraction with carbon tetrachloride yields a pale yellow solid, having the following constants :—Iodine value, 30.4 ; saponification value, 182 ; acid value, 5. It is soluble partially in 95 per cent. alcohol and completely in petrol ether or chloroform. It was separated into an alcohol-soluble and an alcohol-insoluble fraction, the latter being very similar to the lac wax of the incrustation. A detailed investigation of the fat is contemplated and meanwhile the product, which can be easily isolated from the washings of stick lac, has already been used in the preparation of shoe polishes, and finishes for furniture and automobile bodies.

SUMMARY.

1. A preliminary investigation of the physiological products of the lac insect, now running to waste, is presented in this paper. The importance of some of these products in Hindu medicine and their economic value are indicated.

2. A method for isolating the various fractions from brood lac is described in detail.

3. The nitrogenous constituents have been fractionated into an albumin, precipitable by acetic acid or by salt saturation, and a serum mainly consisting of the simpler polypeptides.

4. A Van Slyke analysis of the above two fractions has shown that the albumin is allied in composition to the vegetable globulins while the serum polypeptides have revealed the existence of protamine-like substances closely related to that isolated by Dunn from the

[1] Dunn, M. S., *J. Biol. Chem.*, 1926, **70**, 697.

testicles of *Sardinia*. Attempts to isolate individual polypeptides are in progress.

5. The isolation of the insect-fat by centrifuging, and its subsequent purification are described; the constants point to its being a mixture of a true fat and wax. A detailed analysis of this fat is contemplated.

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[Accepted, 29-3-33.]

STUDIES IN THE PROTEINS OF INDIAN FOODSTUFFS.
PART V. The Alcohol Soluble Protein of Fenugreek (*Trigonella Fœnum Græcum*).

By Y. V. Sreenivasa Rao, B. N. Sastri and N. Narayana.

In a previous communication Sreenivasa Rao and Sreenivasaya (*Jour. Indian Inst. Science*, 1932, **15A**, 122) have reported their findings on the albumin and the globulin of fenugreek. The present paper deals with the prolamin from the same source.*

EXPERIMENTAL.

The flour was first extracted with ether in a Soxhlet and the residue (3 Kg.) stirred up with 70 per cent. alcohol (9 L) in the cold for six hours. The mixture was then warmed on the water bath and maintained under reflux for 1 hour at the boiling temperature. The hot suspension was then filtered and the extract on cooling in the ice chamber deposits a white solid. After filtration, the extract was concentrated at 40° under reduced pressure. The concentrate (2 L) was allowed to stand overnight at room temperature when a white solid separated. The precipitate was washed consecutively with distilled water, 90 per cent. alcohol and ether. It was then dried, powdered to pass through a 100-mesh sieve and preserved in a desiccator over calcium chloride (Preparation I ; yield 5 g.)

On treating the concentrated alcoholic extract (1.5 L) with acetone (8 L), a copious precipitate was obtained a large portion of which was found to be soluble in water. The insoluble portion was separated on the centrifuge, washed repeatedly with distilled water and dehydrated with alcohol and ether. On drying, a light, white solid was obtained (Preparation II ; yield, 4 g.)

Both the above-mentioned preparations gave all the reactions for proteins and contained tyrosine, tryptophan, sulphur and phosphorus. On analysis they gave the following results. (Table I.)

* Since writing this paper a preliminary note on the prolamin of fenugreek prepared by a different method by A. Hassan and M. K. A. Basha has appeared in the *Biochemical Journal* (1932, **26**, 1843.) That publication does not, however, relate to the composition of the prolamin.

TABLE I

Preparation	PERCENTAGES				
	Moisture	Ash	P_2O_5[1]	Ash and moisture free	
				Nitrogen	Sulphur[2]
I	3.0	0.99	0.50	14.03	1.12
II	9.6	1.10	0.41	14.08	0.99

[1] Micro method of Pregl.
[2] Micro-combustion method of Carius.

The two preparations were then analysed by the method of Van Slyke as modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; 1927, **21**, 247). Tyrosine, tryptophan and cystine were estimated separately on the whole protein according to Folin and Marenzi (*J. Biol. Chem.*, 1929, **83**, 89).

The distribution of nitrogen is given in the following table.—

TABLE II

Form of Nitrogen	Percentages of total nitrogen — Preparation I		Percentages of total nitrogen — Preparation II	
Acid insoluble Melanin ...	0.9		1.1	
Acid soluble Melanin ...	0.4		0.5	
Amide ...	20.2		20.0	
Basic ...	5.8		5.6	
Arginine ...		3.1		2.9
Histidine ...		0.7		0.7
Cystine ...		1.4		1.3
Lysine ...		0.7		0.7
Non-basic: ...	72.7		72.8	
Amino ...		70.2		70.4
Non-amino ...		2.5		2.4

A noteworthy feature of the prolamin from fenugreek is its low basic nitrogen content in which respect it is comparable only with eleucine from ragi (Hoffman and Gortner, Coll. Symp. Monograph, 1925, Vol. 2, 246; Niyogi, Narayana and Desai, private communication).

In Table III are given the percentages of the more important amino acids as estimated for the whole protein. The corresponding figures for ragi prolamin are cited for comparison.

TABLE III

	Source of prolamin		Method
	Fenugreek	Ragi	
Arginine ...	2.3	2.5	Direct
Histidine ...	0.4	1.4	Van Slyke
Lysine ...	0.5	0.5	,,
Cystine ...	3.0	2.5	Folin and Marenzi
Tyrosine ...	4.3	5.3	,,
Tryptophan ...	2.4	1.6	,,

SUMMARY.

The two preparations of the prolamin from fenugreek obtained by different methods were found to have very nearly the same composition. The prolamin is characterised by a low basic nitrogen content and high percentages of cystine and tryptophan and thus resembles the alcohol soluble protein of ragi isolated by one of us.

In conclusion, we wish to express our thanks to Professor V. Subrahmanyan for his kind interest in the progress of the work.

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THE MUCILAGE OF FENUGREEK (*TRIGONELLA FŒNUM GRÆCUM*).

By C. R. Harihara Iyer and B. N. Sastri.

In a recent communication on the subject (*Biochem. J.*, 1932, **26**, 255) Daoud has described the mucilage of fenugreek as the silico-phosphoric ester of manno-galactan. His method of preparation consists in the addition of alcohol to an aqueous extract of fenugreek bran till the final concentration of alcohol reaches 30 per cent. The precipitate thus obtained is redissolved in water and reprecipitated by addition of alcohol.

The product so obtained does not however appear to have been subjected to any further purification. The quantitative relationships between phosphorus and silicon or between either of those elements and total carbohydrates have not been examined. The present authors and their associates have been engaged on the chemical examination of fenugreek for the past few years and the object of this communication is to place on record a few important observations on the purification and chemical composition of the mucilage.

Preparation :—The mucilage was first precipitated from the aqueous extract of bran in a manner similar to that described by Daoud (*loc. cit.*). The preparation thus obtained was dissolved in water and re-precipitated by alcohol, the process being repeated several times to obtain a pure product. It was observed that the ash content of the preparation decreased steadily on purification as may be seen from the analyses of successive precipitates (Table I).

TABLE I

Precipitate as obtained in order	Percentages		
	Ash	Phosphorus *	Nitrogen
1	1.57	0.24	...
2	0.68	0.07	0.53
3	trace	0.02	0.11

* According to the micro-method of Pregl.

The results indicate that one or more compounds containing nitrogen and phosphorus occur as impurities in association with the mucilage.

Electro-dialysis.—In order to eliminate crystalloidal impurities mechanically carried down by the mucilage, an aqueous extract of the mucilage (2nd precipitation) was subjected to electro-dialysis using the main laboratory supply (110 D.C.) as the source of current. Three thick arc carbons served as anode and a platinum strip ($1\frac{1}{2}$ sq. in.) was used as cathode. The liquid in the middle cell was periodically stirred, and the dialysis continued for a period of 4 days, when the conductivity reached almost that of distilled water. The product obtained on precipitating the dialysed extract contained the same percentages of phosphorus and nitrogen as the undialysed one, thereby showing that the impurities were not crystalloids.

Saturation with Magnesium Sulphate.—In view of the previous observations of Sreenivasa Rao and Sreenivasaya that phosphorus is associated with the water-soluble protein of fenugreek (*J. Indian Inst. Sci.*, 1932, **15A**, 122), further purification of the mucilage was directed towards obtaining a protein-free product. An aqueous solution of the mucilage was saturated with magnesium sulphate and the precipitate thus obtained separated by centrifuging. The mucilage was precipitated from the centrifugate by addition of alcohol. The precipitate was washed successively with 50 per cent. alcohol, absolute alcohol and finally, ether. The white product thus obtained possessed all the properties of the original mucilage but was entirely free from both phosphorus and nitrogen.

The above observations show clearly that the mucilage itself does not contain any phosphorus or nitrogen but occurs associated with one or more impurities which are either proteins or some other types of compounds that can be removed by protein precipitants.

Removal of impurity by treatment with boiling alcohol.—An aqueous solution of the crude mucilage was treated with alcohol to a concentration of 30 per cent. The suspension thus obtained was refluxed over a boiling water-bath for 8 hours and allowed to stand at the room temperature for 48 hours. The precipitate was then separated, extracted with water and the mucilage precipitated from the aqueous extract with alcohol. Analysis of the precipitate showed that its phosphorus content was almost negligible (0.001 per cent.) thus lending further support to the suggestion that the impurity resembled proteins in behaviour and was denatured and coagulated by boiling alcohol.

Hydrolysis of the mucilage.—On hydrolysis with acids, the mucilage yielded mannose and galactose in equal proportions as has been reported by previous workers. Elementary analysis of the purified product gave the following percentages.—C, 44.3 ; H, 6.2 ; and O, 49.5. The results would thus show that the mucilage is a mannogalactan with the empirical formula, $C_6H_{10}O_5$.

SUMMARY.

The mucilage from fenugreek is usually associated with a nitrogenous impurity which can be removed either by repeated dissolution in water and reprecipitation with alcohol or by saturation with magnesium sulphate. The purified mucilage is a mannogalactan.

The authors' thanks are due to Mr. M. Sreenivasaya for many helpful suggestions.

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CONTRIBUTIONS TO THE STUDY OF THE SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.).

Part XIII. Investigation of the Hexone Bases.

By Y. V. Sreenivasa Rao.

It has been shown by Narasimhamurty and Sreenivasaya (*J. Indian Inst. Sci.*, 1929, **12A**, 153) that with the onset of spike there is distinct increase in the basic nitrogen content of the leaves of sandal. The basic fraction has now been partitioned into individual amino-acid components by Van Slyke's method.

The preparation of the experimental material has been fully described in the previous communication. The leaf material (40 g.) thus obtained was repeatedly extracted with boiling ammonia-free distilled water and filtered. After treatment with 2-3 c.c. of glacial acetic acid, the combined filtrates were passed through paper pulp and the total nitrogen content determined on aliquot portions thereof. The bulk of the filtrate was then hydrolysed with 20 per cent. hydrochloric acid for one hour. After removing the acid-insoluble humin, the hydrolysate was distilled under reduced pressure to remove hydrochloric acid. Calcium oxide was then added in slight excess and the ammoniacal nitrogen distilled under reduced pressure. The residue was filtered with frequent washings and the combined filtrates concentrated to a small volume (50 c. c.).

Hydrochloric acid was added to this solution until the concentration was 1N and 10 grams of phosphotungstic acid were stirred into the liquid. The mixture was then placed on a boiling water-bath until most of the precipitate dissolved. It was taken out and, after cooling to the room temperature, kept in the ice chest for 24 hours. The phosphotungstic acid precipitate was separated by centrifuging and washed repeatedly with ice-cold 1 : 10 hydrochloric acid. The centrifugate and the washings made up to known volume constituted the non-basic fraction. The precipitate was dissolved in the minimum quantity of 1 per cent. sodium hydroxide and made up to known volume.

The basic and the non-basic fractions obtained in the above manner from the four specimens (two healthy and two spiked) from two different areas (Uttarahalli and Ragihalli) were analysed according to the Van Slyke method as modified by Plimmer and his co-workers. (*J. Biol. Chem.*, 1911, **10**, 15 ; *Biochem. J.*, 1925, **19**, 1004 ; *Ibid.*, 1927, **21**, 247). The results are shown in Tables I and II.

TABLE I

Percentages on moisture-free basis.

Form of Nitrogen	UTTARAHALLI AREA		RAGIHALLI AREA	
	Healthy	Spiked	Healthy	Spiked
Total	1.77	2.21	1.94	1.43
Water-soluble ...	0.56	0.78	0.37	0.39
Basic	0.09	0.19	0.08	0.12
Amino ...	0.05	0.10	0.04	0.07
Non-amino ...	0.04	0.09	0.04	0.05
Non-basic	0.38	0.44	0.24	0.28
Amino	0.18	0.20	0.13	0.16
Non-amino ...	0.20	0.24	0.11	0.12

TABLE II

Distribution of nitrogen in the basic fraction.

	PERCENTAGES			
	UTTARAHALLI AREA		RAGIHALLI AREA	
	Healthy	Spiked	Healthy	Spiked
Arginine	66.7	26.3	50.0	33.3
Histidine	3.3	42.1	0.4	16.7
Cystine	22.2	5.3	12.5	25.0
Lysine	11.1	21.1	25.0	25.0

It may be noted that basic fraction forms a greater proportion of the water-soluble nitrogen in the case of the spiked leaves than in the healthy ones. The occurrence of a high percentage of histidine fraction in the diseased condition is also significant and the necrosis of the root ends of the diseased plant is explained by the assumption that histidine is converted into histamine. Attempts to detect histamine in the diseased tissues have not, however, so far been successful.

SUMMARY.

The distribution of hexone bases in the water extract from the leaves of healthy and spiked plants from two different sources has been determined.

It has been observed that the basic nitrogen content of the spiked leaf is greater than that of the healthy one. Particular mention should be made of the histidine fraction which occurs in large quantities in the former while being present only in small amounts in the latter.

My thanks are due to Mr. M. Sreenivasaya for many useful suggestions.

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[Accepted, 3-5-33.]

CONTRIBUTIONS TO THE STUDY OF THE SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.).

Part XIV. Study of Mosaics associated with spiked areas.

By Y. V. Sreenivasa Rao.

It was observed in a number of spiked areas that many of the weeds like Aegeratum and Gysekia Spp. were affected with mosaic. The absence of this disease from healthy areas was rather striking and in view of its similarity to and possible association with the incidence and spread of sandal spike, an investigation of the mosaic of aegeratum was undertaken. The present paper relates to the distribution of inorganic constituents and nitrogen metabolism in conditions of health and disease.

The inorganic constituents were estimated according to the A.O.A.C. (1925) methods. Nitrogen distribution was determined by the method of Van Slyke modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; *Ibid.*, 1927, **21**, 247). The results have been presented in Tables I and II respectively.

TABLE I

Expressed as percentages on moisture-free material.

	AREA			
	JAVALGIRI		AIYUR	
	Healthy	Diseased	Healthy	Diseased
Total nitrogen ...	3,92	4.53	4.65	.3.40
Phosphoric acid (as P_2O_5)	0.78	0.43	1.88	0,89
Calcium (as CaO) ...	3.97	3.74	3.14	2.45

TABLE II

Percentages of total nitrogen.

	AREA			
	JAVALGIRI		AIYUR	
	Healthy	Diseased	Healthy	Diseased
Insol. Humin	7.84	7.92	2.53	7.46
Soluble Humin	1.91	1.42	0.78	1.23
Amide N*	8.91	12.55	5.88	9.07
Free ammonia* ...	1.2	3.4	0.9	2.8
Total basic	13.84	14.53	16.50	19.73
Non-basic	60.10	60.50	67.89	61.18
Amino	48.67	56.0	65.33	57.51
Non-amino ...	11.39	4.5	2.56	3.67

* Estimated according to Grafe's method (*Z. Physiol. Chem.*, 1906, 48, 300).

Inorganic constituents (Table I) do not show any striking differences. Tissues of spiked sandal are characterised by a low content of calcium while aegeratum affected with mosaic contains about the same percentage of calcium as the corresponding healthy leaves. Diseased aegeratum leaves have, on the other hand, lower phosphorous contents than the healthy ones.

The distribution of nitrogen (Table II) shows that the disease is more allied to tobacco mosaic and spinach blight than to sandal spike, if the ammoniacal nitrogen is taken into consideration. There is a striking increase in the ammoniacal nitrogen in the diseased aegeratum leaves, while in the case of the spike-disease of sandal the ammoniacal nitrogen is not appreciably affected. The higher basic nitrogen characterising spiked sandal is absent from the diseased aegeratum.

The author's thanks are due to Professor V. Subrahmanyan for his keen interest in this work.

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[Accepted, 3-5-33.]

PHYSIOLOGICAL PRODUCTS OF THE LAC INSECT

PART II. INVESTIGATION OF THE WATER SOLUBLE NITROGENOUS CONSTITUENTS

By N. K. Ranga Rao.

In a previous communication (*J. Indian Inst. Sci.*, 1933, **16A**, 77) it has been shown that the water soluble nitrogenous bodies not precipitated by acetic acid, alcohol or salt solution, consisted of the simpler polypeptides, among which a protamine-like body with a high percentage of basic nitrogen was found. The present investigation deals with the fractionation of the serum polypeptides with the object of isolating simpler mixtures if not individual compounds.

Several modifications in extraction and treatment were adopted with the object of obtaining the polypeptides, as far as possible, in their native state and without any admixture of foreign bodies. Thus, the extraction of the material was carried out with distilled water instead of with 0.9 per cent. salt solution. Concentration of the aqueous extract was effected by freezing out the water. The proteins were precipitated directly with alcohol. The following is a schematic representation of the treatment.

INCRUSTATION (35 lbs.)

(Extracted with 10 litres of water, concentrated to 3 litres and treated with 5 vols. of alcohol)

- Precipitate (1)
- Filtrate (Concentrated to 3 litres and barium hydroxide solution added)
  - Precipitate (2) (Colouring matter)
  - Filtrate (Carbon dioxide passed)
    - Precipitate (3) (Barium carbonate)
    - Filtrate (Treated with phosphotungstic acid solution)
      - Precipitate (Extracted with barium hydroxide solution and CO_2 passed)
        - Precipitate (4) (Barium phosphotungstate and carbonate)
        - Filtrate (Concentrated to a syrup and alcohol added)
          - Precipitate (5)
          - Filtrate (6) (Concentrated and dried)
      - Filtrate (Treated with barium hydroxide solution and CO_2 passed)
        - Precipitate (7) (Barium phosphotungstate and carbonate)
        - Filtrate (Concentrated to 500 c. c.)
          - Precipitate (8)
          - Filtrate (Concentrated to a syrup and alcohol added)
            - Precipitate (9)
            - Filtrate (Concentrated to a syrup and extracted with butyl alcohol)
              - Butyl alcoholic extract (10)
              - Solution (Treated with phosphotungstic acid solution)
                - Precipitate (11)
                - Filtrate (12)

Fractions 1, 2, 3, 4 and 7 were dried at 97° and bottled. Numbers 3, 4 and 7 were white powders: 1 and 2 were dark red in colour. Fraction 8 crystallised in the form of fine, white needles. Fractions 5 and 9 were obtained as brownish powders. They were washed thoroughly with absolute alcohol and preserved in a vacuum desiccator. On removing alcohol under reduced pressure, fractions 6 and 10 yielded brown powders which were also preserved in the vacuum desiccator.

Results.—The nitrogen contents of the different fractions were estimated.

TABLE I

Fraction No.	Total Nitrogen content in gms.	Percentage on total water soluble nitrogen	Weight of substance gms.	Percentage of nitrogen in the preparation
1	1·50	16·48	...	...
2	0·26	2·86	63·0	0·41
3	0·06	0·66	8·0	0·75
4	0·21	2·31	40·0	0·05
5	0·20	2·20	2·5	11·41*
6	3·40	37·37	23·2	14·67
7	0·25	2·75	140·0	0·18
8	0·23	2·53	3·1	7·60
9	0·87	9·56	28·0	4·30*
10	1·42	15·61	15·0	9·47
11	0·30	3·30	...	...
12	0·31	3·41	...	...
	9·01	99·04		

* On ash-free sample.

TABLE II

Nitrogen distribution.

	Fraction 5	Fraction 6	Fraction 9	Fraction 10
Humin N ...	8·44	5·08	5·43	4·52
Acid amide N ...	2·62	13·02	2·62	2·82
Basic N ...	47·89	41·67	16·55	25·80
Arginine N ...	18·73	18·05	12·29	19·03
Histidine N ...	30·35	19·43	4·31	8·87
Lysine N ...	Nil	4·19	Nil	Nil
Cystine N ...	...	...	...	...
Non-basic N ...	39·20	39·93	75·43	66·94
Amino N in basic form ...	13·60	15·20	4·47	5·61
Amino N in nonbasic form ...	40·12	33·57	40·63	28·07

TABLE III

Properties of the nitrogenous compounds.

	FRACTION				
	5*	6	8	9*	10
Solubility in water.	Soluble	Readily soluble	Soluble in hot water	Readily soluble	Readily soluble
Solubility in alcohol.	Soluble	Soluble	Insoluble	Soluble	Soluble
Solubility in butyl alcohol.	Soluble	Insoluble	Insoluble	Soluble	Soluble
Phosphotungstic acid.	Precipitate	Precipitate	No precipitate	Precipitate in acid medium	Precipitate in acid medium
Mercuric acetate.	Precipitate	Precipitate	No precipitate	Precipitate	Precipitate
α-naphthol test (Molisch test).	–	+	–	–	+

* Freed from metallic radical.

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Discussion of Results.—Fraction 1 is a mixture of proteins and peptones which are precipitated with 5 volumes of alcohol. Since fraction 2 is made up chiefly of the lac dye, which is known to be free from nitrogen, it is to be inferred that 0.26 gm. of nitrogen found in that fraction (63 gms.) may be regarded as having been occluded. Fractions 3, 4 and 7 are barium salts of phosphotungstic and carbonic acids and they have in all 0.52 gm. of nitrogen precipitated with them. Fractions 5 and 6 are nitrogenous bodies precipitated by phosphotungstic acid, but fraction 5 differs from fraction 6 in that it forms a compound with barium hydroxide and is soluble in butyl alcohol. Fractions 9 and 10 are nitrogenous bodies not precipitated by phosphotungstic acid, but fraction 9 differs from fraction 10 since it forms a hygroscopic compound with barium hydroxide. The nitrogenous bodies not extracted by butyl alcohol are represented by fraction 12.

A detailed investigation of the fractions which had either a high percentage of nitrogen or contained comparatively large quantities of nitrogen was carried out.

Fraction 8.—This is a white needle-shaped crystalline substance soluble in hot water. It has been identified as tyrosine by its combustion values, nitrogen content, amino nitrogen content and Millon's reaction. (Found : C, 59.0 ; H, 6.10 ; N, 7.68. $C_9H_{11}O_3N$ requires C, 59.64 ; H, 6.12 ; N, 7.74 per cent.)

Fractions 5, 6, 9 *and* 10.—The distribution of nitrogen in these compounds has been determined by the Van Slyke method. Fraction 5 contains 47.9 per cent. of basic nitrogen of which 30.4 per cent. is histidine and 18.7 per cent. arginine. The ash content of this fraction is 29.8 per cent. About 32 per cent. of the total water soluble nitrogen separated as fraction 6 which contains 42 per cent. of basic nitrogen of which 18 per cent. is arginine and 19, histidine. The non-basic nitrogen of compounds of fractions 5 and 6 occur almost entirely in the amino form. Fractions 9 and 10 are poor in the basic fraction and are not therefore readily precipitated by phosphotungstic acid. The former forms a stable compound with barium hydroxide from which the free polypeptide cannot be obtained by passage of carbon dioxide as is usually the case but has to be treated with sulphuric acid to remove barium. The non-basic nitrogen content of this compound is very high, being 75.4 per cent. Its ash content is 21.8 per cent. Further work on this compound is in progress. Fraction 10 is neither precipitated by phosphotungstic acid, nor forms a compound with barium hydroxide. The nitrogen distribution of this compound is given in Table II.

SUMMARY

1. The nitrogenous bodies of the lac wash liquor have been separated into four fractions, two of which are precipitated by phosphotungstic acid. These fractions are rich in basic nitrogen and constitute about 35 per cent. of the total water soluble nitrogen. The other two fractions not precipitated by phosphotungstic acid are rich in the non-basic fraction (75 per cent.) and constitute about 25 per cent. of the total nitrogen.

2. Free tyrosine has been obtained from the body fluids of the lac insect. It has been found to be present to the extent of 2.5 per cent. on the total water soluble nitrogen.

My grateful thanks are due to Mr. M. Sreenivasaya and Professor V. Subrahmanyan for their many helpful suggestions in the course of this investigation.

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EXTENSION OF MICHAEL'S REACTION PART III

By Tejendra Nath Ghosh and Praphulla Chandra Guha.

Schlenk, Appenrodt, Michael and Thal (*Ber.*, 1914, **47**, 473) have studied the action of compounds containing the unsaturated systems C=N, N=N with sodium in ethereal solution, and the structure of the additive compounds established from a study of the products obtained by the action of water and carbon dioxide. There is no record of work in which unsaturated compounds of the above type have been made to react with the sodium derivatives of organic compounds containing active methylene groups.

In parts I and II of this series (*J. Indian Chem. Soc.*, 1930, **7**, 264-273), the action of sodium derivatives of urethane on unsaturated esters, isocyanates and isothiocyanates has been studied. The work has now been further extended to determine whether unsaturated systems like N=N, C=N, would lend themselves to ordinary Michael's condensation in forming additive compounds with sodium derivatives of acetoacetic ester, cyanacetic ester, acetylacetone and urethane.

Phenyl azo-carboxylic ester reacts with the sodium derivatives of ethyl acetoacetate, ethyl cyanacetate and acetylacetone to yield β-N-carbethoxyacetylmethyl-β-phenylcarbazinic ester (I), β-N-carbethoxycyanomethyl-β-phenylcarbazinic ester (II) and β-N-diacetylmethyl-N-phenylcarbazinic ester (III) respectively.

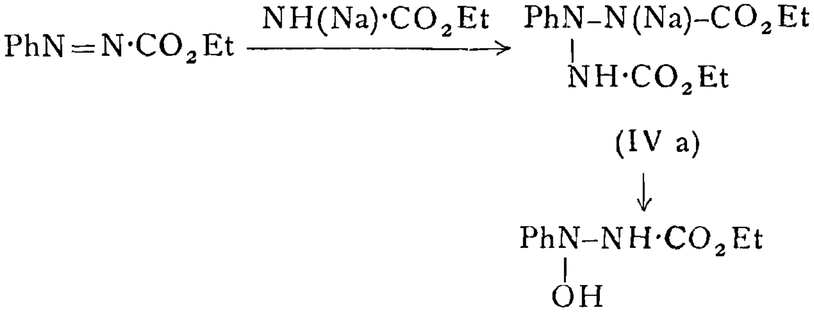
$$PhN=N-CO_2Et \xrightarrow{CH_3CO{\cdot}CHNa{\cdot}CO_2Et} \begin{array}{l} NPh-NNa-CO_2Et \\ | \\ CH(COCH_3){\cdot}CO_2Et \end{array}$$

$$\begin{array}{ccc} PhN-NH{\cdot}CO_2Et & PhN-NH-CO_2Et & PhN-NH-CO_2Et \\ | & | & | \\ CH(COCH_3){\cdot}CO_2Et & CH(CN){\cdot}CO_2Et & CH(COCH_3)_2 \\ \text{(I)} & \text{(II)} & \text{(III)} \end{array}$$

The yields of these compounds are generally very poor. They are unstable in presence of cold dilute alkali and hot acid. Decomposition of compound (I) into phenyl isocyanide on hydrolysis further confirms the direct attachment of the acetoacetic ester grouping with the β-nitrogen atom.

Ethyl phenylazocarboxylate reacts with the sodium derivative of urethane to form an unstable intermediate product (IVa) which, in

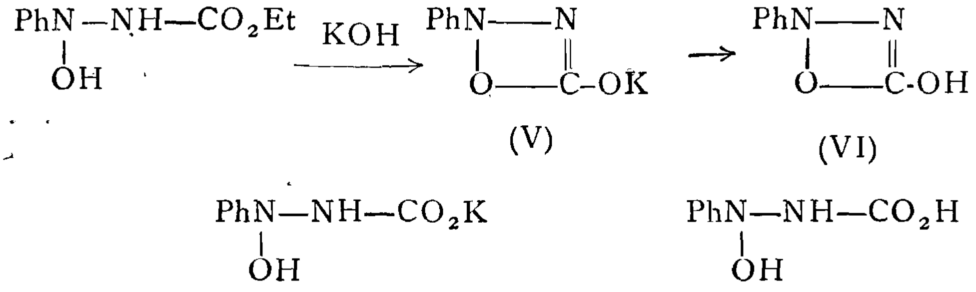
presence of acids, gets readily hydrolysed into urethane and β-N-hydroxy-β-phenylcarbazinic ester (IV).



(IV a)

(IV)

On treatment with aqueous alkali compound IV yields the sparingly soluble potassium salt (V) from which acids liberate the compound VI.



(V) (VI)

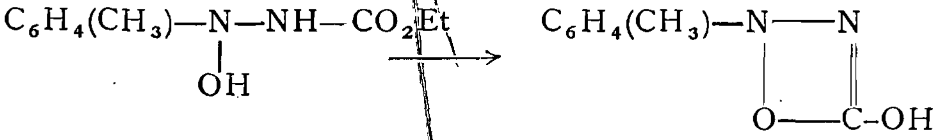
(V a) (VIa)

Though compounds (V) and (VI) with their water of crystallisation agree with the composition of potassium-β-hydroxyphenyl carbazinate (PhN(OH)-NH-CO_2K) and the carbazinic acid, respectively, the ring structure (V) and (VI) have been ascribed on the basis of the following facts : (*a*) compound (VI) cannot be esterified, (*b*) on treatment with ethyl iodide it gives the compound (VII) and *not* (IV), (*c*) the corresponding tolyl substituted compound (VIII) does not contain any water of crystallisation and hence cannot possess an open chain structure like (VI a), and (*d*) though the potassium salts and esters of carbazinic acid and substituted carbazinic acids are known to exist, no such free acid is known.

The compound (V) gives with ethyl iodide and methyl iodide the corresponding ethyl and methyl derivatives (VII) and (VIIa) respectively :—

(VII) (VIIa)

Ethyl *p*-tolylazocarboxylate reacts similarly with the sodium derivative of urethane to yield the compound (VIII), the intermediate product (VIII a) could not, however, be isolated.



(VIIIa) (VIII)

Ethyl azodicarboxylate $CO_2Et\text{-}N=N\text{-}CO_2Et$ with the sodium derivative of acetoacetic ester gives similarly N-carbethoxyacetylmethylhydrazodicarboxylic ester (IX).

$$CO_2Et{\cdot}N=N{\cdot}CO_2Et \xrightarrow{CH_3CO{\cdot}CHNa{\cdot}CO_2Et} CO_2Et{\cdot}N(CHAc{\cdot}CO_2Et){\cdot}NH{\cdot}CO_2Et$$

(IX)

The conclusion to be drawn from the inactivity of azobenzene in such reactions is that for Michael's condensation with systems like (-N=N-), it is essential that there should be at least one strongly negative group attached to one of the nirtogen atoms. An analogous observation was made by Ingold and Weaver (*J. C. S.*, 1925, **127**, 378) about the inactivity of the ketens in their addition to azobenzene though the addition took place quite readily with phenylazocarboxylic ester and azodicarboxylic ester. Ingold's explanation of this difference which is based on the assumption that the carbethoxy group has much smaller affinity demand than phenyl, is equally applicable to the present instance.

It was next contemplated to study Michael's condensation with compounds containing the unsaturated grouping (-N=C-) and differently substituted by the negative groups phenyl and carboxyl e.g. compounds like (i) PhN=CHPh, (ii) $PhN=CH{\cdot}CO_2Et$, (iii) $CO_2Et{\cdot}N=CHPh$,

(iv) $PhN{=}C(CO_2Et)_2$, (v) $CO_2Et{\cdot}N{=}CH{\cdot}CO_2Et$, (vi) $CO_2Et{\cdot}N{=}C(CO_2Et)_2$. Of these above six types,[1] only compounds i and ii are known. They do not lend themselves to ordinary Michael's condensation. The inactivity of phenylazomethinecarboxylic ester as distinct from the reactivity of phenylazocarboxylic ester is perhaps due to the group (-N=CH-) inducing some basic character to the former.

As it was not possible to study Michael's condensation with compounds of type (iv), no such compound being known in literature, the addition reaction of mesoxalic ester phenylhydrazone, in which, like (iv), there are two negative (CO_2Et) groups attached to the unsaturated carbon atom, has been studied. This reacts with the sodium derivative of ethyl- acetoacetate, malonate and cyanacetate yielding additive compounds all of which get easily hydrolysed and suffer disruption giving the compound (X).

$$PhNH{\cdot}N{:}C(CO_2Et)_2 \xrightarrow{CH(Na)R{\cdot}CO_2Et} PhNH{\cdot}N{\cdot}CNa(CO_2Et)_2 \text{ (with } CHR{\cdot}CO_2Et \text{ on N)}$$

$$\xrightarrow{\text{acid}} \underset{(X)}{PhNH{-}NH{-}CH(CO_2Et)CO_2H} \quad (R = CO_2Et,\ CN \text{ or } COCH_3).$$

$$\underset{(XI)}{PhN{=}N{-}CH(CO_2Et){\cdot}CO_2H} \qquad \underset{(XII)}{PhNH{-}NH{-}CH_2{-}CO_2H}$$

The structure of (X) is confirmed by the following facts: (1) against alkali it titrates as a monobasic acid, (2) it is oxidised by ferric chloride to an azo-derivative (XI), (3) it gets easily hydrolysed by alcoholic potash to phenylhydrazine acetic acid (XII).

It was planned at this stage to utilise the applicability of Michael's reaction with mustard oils and isocyanates (cf. Ghosh and Guha, *J. Indian Chem. Soc.*, 1930, **7**, 165) for the synthesis of pyridine derivatives. Carbethoxythiocarbimide was accordingly condensed with the

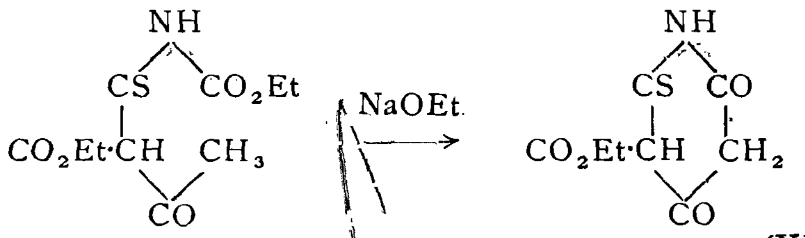
$$CO_2Et{\cdot}N{:}C{:}S \xrightarrow{CH_3CO{\cdot}CHNa{\cdot}CO_2Et} SC\begin{cases} N(Na){\cdot}CO_2Et \\ CHAc{\cdot}CO_2Et \end{cases} \xrightarrow{\text{acid}} \underset{(XIII)}{SC\begin{cases} NH{\cdot}CO_2H \\ CHAC{\cdot}CO_2Et \end{cases}}$$

---

[1] Attempts are being made to prepare compounds of the type (III) to (VI) in this laboratory.

sodium derivative of acetoacetic ester resulting in the formation of thio-carbethoxy-aceto-acetyl carbamic acid (XIII).

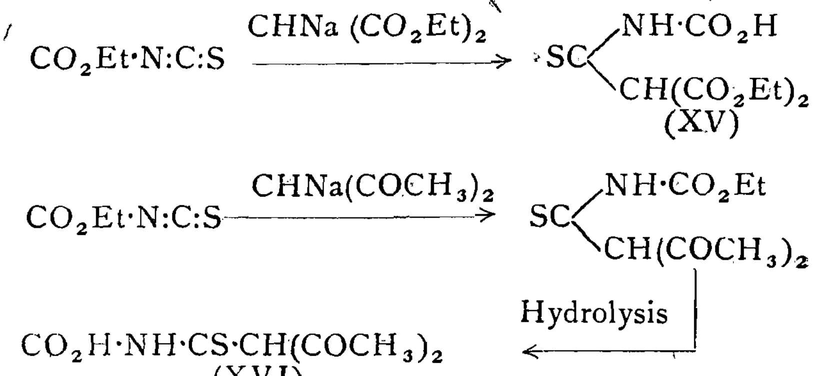
The acid (XIII), after esterification was treated with molecular proportion of sodium ethylate with the hope of getting the pyridine compound (XIV) by elimination of a molecule of alcohol (cf. Scheiber and Miessel; *Ber.*, 1915, **48**, 238; Mayuranathan and Guha, *J. Indian Inst. Sci.*, 1933, **15A**, 131);



(XIV)

but, our expectation has not been fulfilled, as from the reaction mixture, only the original ester and a very small quantity of red viscous liquid could be isolated.

Compound (XIII) as also those obtained from acetylacetone and ethyl malonate, contain a free carboxyl group. It is not easy to locate definitely the position of this group in these compounds; still, by analogy with compound (XVI) in which the COOH group must have been derived from the CO_2Et attached to nitrogen—the conversion of one of the acetyl groups into a free carboxyl being unlikely—the free carboxyl groups in compounds (XIII) and (XV) have been shown attached to the end nitrogen atoms.



(XV)

(XVI)

sodium salt gave on acidification a tarry mass which on ether treatment yielded a small quantity of brown solid crystallising from alcohol in colourless plates; m.p. 168-170°. It is soluble in cold dilute alkali and is precipitated unchanged by acids (Found: N, 17.32. $C_8H_8O_2N_2$ requires N, 17.07 per cent.).

β-N-carbethoxyacetylmethylhydrazine dicarboxylic ester (IX).—Azodicarboxylic ester (5.8 g.; prepared according to the method of Ingold *J. C. S.*, 1925, **127**, 381) was added to the sodium derivative of acetoacetic ester (5.1 g.) in ethereal suspension and the mixture allowed to stand for a day. An aqueous solution of the separated sodium derivative on acidification with dilute hydrochloric acid, gave a semi-solid which crystallised from benzene; m.p. 74-75°; yield 5 g. (Found: N, 9.03. $C_{12}H_{20}O_7N_2$ requires N, 9.21 per cent.).

Mesoxalic esterphenylhydrazone and acetoacetic ester: Formation of phenylhydrazine malonic mono ester (X).—The aqueous solution of the reaction product, on acidification, gave a precipitate which crystallised from alcohol in beautiful yellowish needles, m.p. 110-111°. It is soluble in sodium bicarbonate solution and is precipitated unchanged by acids (Found: N, 11.93; M.W. by titration, 235. $C_{11}H_{14}O_4N_2$ requires N, 11.76 per cent.; M.W., 238).

The compound (m.p. 110-111°) obtained from mesoxalic esterphenylhydrazone and the sodium derivative of malonic ester or cynacetic ester, was proved to be identical with compound (X) by the mixed melting point method and also by analysis.

The *azo-compound* (XI).—Compound (X) on being boiled with aqueous ferric chloride during two hours gave a red precipitate which crystallised from alcohol in reddish-brown prisms, m.p. 174-176° (Found: N, 11.62. $C_{11}H_{12}O_4N_2$ requires N, 11.86 per cent.).

Hydrolysis of compound (X): *Formation of compound* (XII).—The compound (X) was heated under reflux with 15 per cent. alcoholic potash for about half-an-hour when the yellowish potassium salt separated out. It was dissolved in water and acidified with dilute hydrochloric acid when a yellow solid was obtained crystallising from alcohol in yellow needles; m.p. 152-153°. It is soluble in aqueous sodium bicarbonate (Found: N, 16.51. $C_8H_{10}O_2N_2$ requires N, 16.86 per cent.). Its identity with phenylhydrazinoacetic acid (m.p. 153°) (*Monatsh.*, **17**, 631) was established.

The *phenylhydrazine* salt was obtained by adding phenylhydrazine (1 mol.) to an ether solution of the above compound (X, 1 mol.). The white precipitate crystallised from ether in colourless plates, m.p.

124-125° (Found: N, 16.52. $C_{17}H_{22}O_4N_4$ requires N, 16.18 per cent.).

Carbethoxythiocarbimide and acetoacetic ester: Formation of thiocarbethoxyacetoacetylcarbamic acid (XIII).—The aqueous solution of the sodium derivative prepared in the usual manner, gave on acidification, a reddish yellow heavy liquid. For purification, a solution of the oil in sodium bicarbonate after being thoroughly shaken with ether was acidified with hydrochloric acid when a yellowish oil separated (Found: S, 13.44; M.W., 230. $C_8H_{11}O_5NS$ requires S, 13.73 per cent.; M.W., 233). The liquid decomposes on slow heating even under reduced pressure.

The *ethyl ester* was obtained by boiling an absolute alcoholic solution of the above acid with a small quantity of concentrated sulphuric acid during eight hours. The solution was then neutralised with calcium carbonate, and the precipitate filtered off. The clear solution after removal of alcohol was poured into water; the separated oil was washed with water and then ether extracted. The ethereal solution yielded a light brown mobile liquid which could not be distilled without decomposition (Found: S, 11.82. $C_{10}H_{15}O_5NS$ requires S, 12.26 per cent.).

Attempt to effect ring closure of the above ester.—An alcoholic solution of the ester (1 mol.) was heated under reflux for about 12 hours with sodium ethoxide (1 mol.), the alcohol distilled under reduced pressure and the solid residue dissolved in water. The aqueous solution, on acidification, yielded a reddish liquid, the alkali-insoluble portion of which was identified to be the original ester. The alkaline solution, on acidification, gave a very small quantity of reddish viscous liquid insoluble in sodium bicarbonate. It did not solidify in a vacuum desiccator on long standing. It contained nitrogen and sulphur.

Thiodicarbethoxyacetylcarbamic acid (XV).—A toluene solution of carbethoxythiocarbimide (7 g.; prepared according to the method of Doran, *J. C. S.*, 1896, **69**, 326) was added to an ether suspension of sodiomalonic ester obtained from malonic ester (8 g.) and sodium (1.15 g.) and the mixture allowed to stand overnight. Most of the separated white precipitate dissolved readily in water leaving a small portion undissolved. The aqueous solution, on acidification gave a precipitate which crystallised from alcohol in yellowish prisms m.p. 70-72°; yield 6 g. It is soluble in sodium bicarbonate solution and is precipitated unchanged by acids (Found: S, 11.92; M.W. by titration, 261. $C_9H_{13}O_6NS$ requires S, 12.16 per cent.; M.W., 263).

The water insoluble portion crystallised from acetic acid in beautiful colourless plates; m.p. 164-165°. It contains sulphur but no

nitrogen. The compound could not be further studied due to its extremely poor yield.

Thiodiacetylacetocarbamic acid (XVI).—The aqueous solution of the sodium derivative on acidification, gave a yellow precipitate which crystallised from dilute alcohol in yellow needles, m.p. 121-122° (decomp.) Found : S, 15.45; M.W. by titration, 201. $C_7H_9O_4NS$ requires S, 15.76 per cent.; M.W., 203).

Thiocarbethoxycyanoacetylcarbamic ester (XVII).—The aqueous solution of the sodium derivative gave on acidification, a semi-solid which on keeping turned into a crystalline product. It crystallised from acetic acid in colourless prisms m.p. 152-153° (decomp.) (Found : N, 11.14. $C_9H_{12}O_4NS$ requires N, 11.47 per cent.). It is insoluble in alkali.

Hydrolysis of (XVII) *into thiocarbethoxycyanoacetylcarbamic acid* (XVIII).—A mixture of the above ester (3 g.) and N/10 caustic potash solution (50 c.c.) was allowed to stand for two days and then acidified with dilute hdyrochloric acid; the resulting precipitate crystallised from alcohol in colourless prisms m.p. 158-160° (decomp.) (Found : N, 12.62; M.W. by titration, 214. $C_7H_8O_4N_2S$ requires N, 12.96 per cent.; M.W., 216).

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STUDIES IN BRIDGE FORMATION

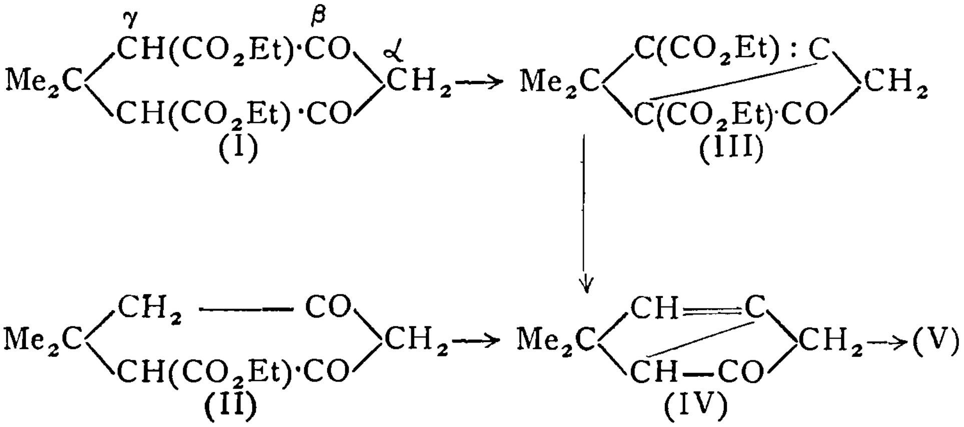
PART III. THE INFLUENCE OF THE METHYL AND CARBETHOXY-GROUPS IN THE INTERNAL DEHYDRATION OF *gem*-DISUBSTITUTED DIHYDRORESORCINOL ESTERS

BY P. S. MAYURANATHAN.

Since the classical researches of Crossley and his co-workers on the conversion of dimethyldihydroresorcinol to chloro-*ortho*xylenes with phosphorus pentachloride (*J. C. S.*, 1903, **83**, 110, 494), no attempt has been made to explain this phenomenon as a process of internal dehydration and subsequent redistribution of the valencies to the aromatic complex. It is the endeavour of the author to elucidate this phenomenon by the application of a method discovered in an earlier paper (Mayuranathan and Guha, *J. Indian Inst. Sci.*, 1932, **15**A, 131) wherein a dehydrated form of the resorcinol, $C_8H_{10}O$ (IV), was isolated during hydrolysis of Scheiber and Miesel's ester (I) (*Ber.*, 1915, **48**, 238), which has now been utilised in determining the characteristics of other substituted dihydroresorcinol esters.

Scheiber and Miesel state that strong baryta hydrolyses the ester (I) to the corresponding dihydroresorcinol, $C_8H_{12}O_2$ (V). This transformation has been shown in the preceding paper (*loc. cit.*) to be only a sequel to the formation of a bridged *cyclo*hexenone, $C_8H_{10}O$ (IV), formed by dehydration of the ester during hydrolysis which is later transformed into dimethyldihydroresorcinol. A constitution was tentatively proposed to the bridged *cyclo*hexenone based on its ease of formation from Scheiber and Miesel's ester in contrast with its non-formation from Vorländer's ester (II) (*Annalen*, 1896, **194**, 257). As mentioned therein, in differentiating the characteristics of Scheiber and Miesel's ester from that of Vorländer's, enolisation in the $\beta\gamma$ position was assumed in the former; still this cannot leave out of account the possibility of the latter behaving similarly, at any rate in a lesser degree of magnitude. In justification, it can be stated that, concurrently with the formation of dimethyldihydroresorcinol during hydrolysis of (II), the ketone (IV) is also formed. The same product has been obtained with extreme difficulty and only in traces from dimethyldihydroresorcinol by the action of phosphorus pentoxide. These

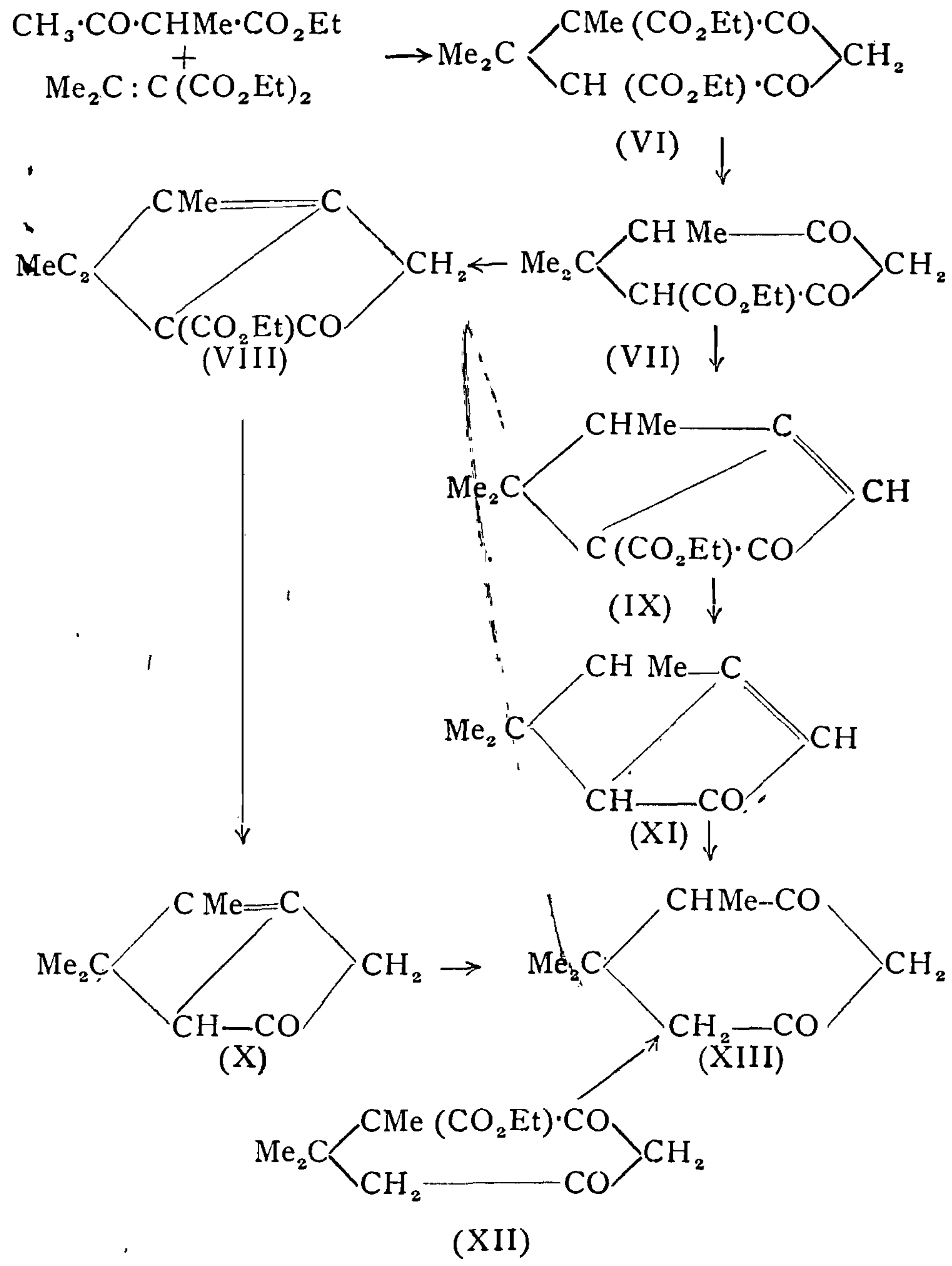
observations appear to warrant the statement that some unique characteristics have to be attributed to the *gem*-disubstituted dihydroresorcinol residue.



The chief difficulty lies, however, in precisely explaining the mechanism of dehydration, since a hydroxyl group can be formed by enolisation in two different ways, although the one presumed is more probable from a comparison of properties of (I) and (II). Furthermore, in illustration of the hypothesis that dehydration precedes decarboxylation in the formation of the new ketone two dehydrated esters (III) and (VIII) have been isolated from (I) and (VII) respectively, which in their turn have been converted to the corresponding ketones (IV) and (X). Thus the constitution of the bridged *cylo*hexenone having gained some support, it seemed desirable to extend the work to similar esters (VII) and (XIV) having additional methyl groups to further facilitate dehydration in analogy with Rothstein and Shoppee's observation (*J. C. S.*, 1927, 531) regarding the influence of the methyl groups on the lactolone formation in the α-ketoglutaric acids.

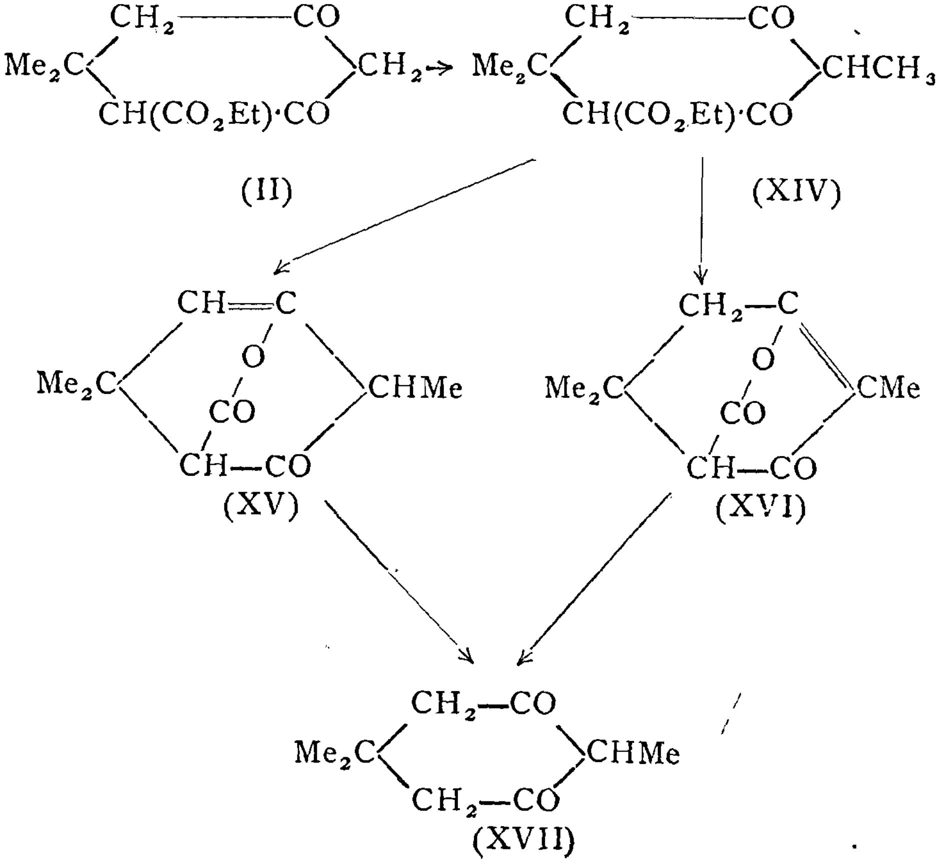
The principal product obtained from *iso*propylidene malonic ester and methyl acetoacetic ester is ethyl *cyclo*hexane-1 :1 :2-trimethyl-3 :5-diketo-6-carboxylate (VII). The diester (VI) as soon as it is formed loses one carbethoxy-group and in analogy with the observations of Crossley (*J.C.S.*, 1909, **95**, 19; 1910, **97**, 520) the elimination is likely to take place from the carbon atom carrying the methyl group. Moreover, as this dihydroresorcinol ester is different from its isomer (XII) obtained by Crossley (*ibid*, 1901, **79**, 138) from mesityl oxide and methyl malonic ester, the constitution (VII) assigned to the ester seems to be quite probable. The structure gets further support from the identity of (VII) with the product obtained from methyl mesityl oxide and malonic ester (cf. Bardhan, *J. C. S.*, 1928, 2608).

Unlike Vorländer's ester the present one is converted comparatively easily to its dehydrated ester (VIII) or (IX) which is finally hydrolysed to a methylated ketone $C_9H_{12}O$ (X) or (XI). Formulae (VIII) and (X) for the dehydrated ester and the ketone have been preferred (the



closely assembled methyl groups being undoubtedly the controlling factors) in analogy with the formation of the ketone, $C_8H_{10}O$; any comparison with (VI) and (I) being impossible as the activity of the former has been greatly impaired by the substitution of an active hydrogen with a methyl situated propinquous with *gem*-dimethyl group. On boiling with baryta or keeping with strong alkali, the ketone (X) is converted into the trimethyldihydroresorcinol (XIII) (Crossley, *loc. cit.*).

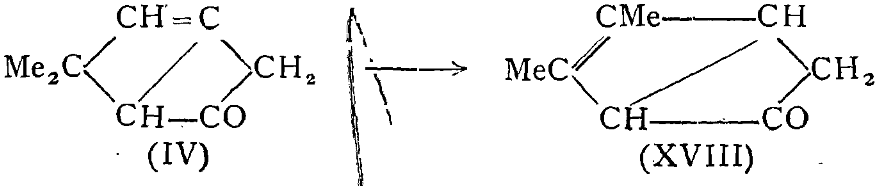
Methylation of Vorländer's ester or Scheiber and Miesel's ester gives ethyl *cyclo*hexane-1 :1 :4-trimethyl-3 :5-diketo-6-carboxylate (XIV), proof for constitution being its conversion on hydrolysis into the trimethyldihydroresorcinol (XVII) (Desai, *J. C. S.*, 1932, 1079). On hydrolysing with sulphuric acid an appreciable quantity of a stable, crystalline solid, $C_{10}H_{12}O_3$, m.p.79°, is obtained along with the corresponding dihydroresorcinol (XVII). With hot alkali it titrates as a dibasic acid and so it is an internal lactone (XV) or (XVI) formed evidently by dehydration in the enolic form of the acid corresponding to the ester (XIV). This reaction seems to be remarkable and is



perhaps a new example of bridged-lactone formation and indicates a fulfilment of Vorländer's expectation (*loc. cit.*) with his ester.

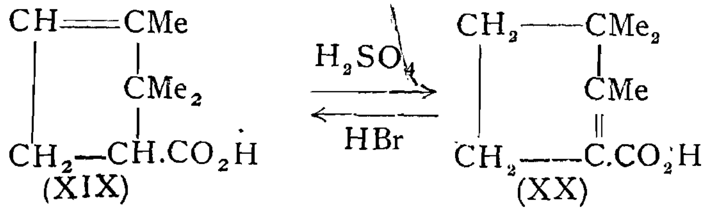
A noteworthy feature of these reactions is the influence exercised by the third methyl group besides the two already present in ester (II) in giving an appreciable yield of the lactone (XV or XVI) and the bridged ketone (X) from the esters (XIV) and (VII) respectively.

It will be observed that compounds (IV), (VIII), (IX), (X), (XI), (XV) and (XVI) do not conform with Bredt's hypothesis, which postulates that a double bond cannot be stable adjacent to the bridged head of a bridged six-membered ring (Bredt, Thuoet, and Schmitz, *Annalen,* 1924, **437**, 1; Windaus and Bohne, *ibid.*, 1925, **442**, 7) and it is possible that a wandering of the methyl group occurs, thus:—



A similar example is the conversion of camphenilol to santene by Ruzicka and Leibl (*Helv. Chim. Acta*, 1923, **6**, 271).

This assumption, however, appears improbable because of the conversion of the bridged *cyclo*hexenone to dimethyldihydroresorcinol which requires a reversal of (XVIII) to the phase (IV). Although Wagner-Meerwein rearrangement of a reversible type has been established in the conversion of *a*-campholytic acid (XIX) to *iso*lauronolic acid (XX) and *vice versa* (Noyes, *Ber.*, 1895, **28**, 548; *Amer. Chem. J.*, 1895, **17**, 428; Perkin, *J. C. S.*, 1903, **83**, 854; Walker and Cormack, *J. C. S.*, 1900, **77**, 378)



the mechanism cannot readily be applied in the case of the bridged ketones or lactone. The possibility of a rearrangement taking place must therefore be left undecided until the constitution of the bridged products is definitely established in attainment of which work is in progress.

EXPERIMENTAL

Hydrolysis of Vorländer's ester (II) *to bridged* cyclo*hexenone* (IV), with (*a*) *cold alcoholic potash*.—The ester (100 g.) was mixed with 25 per cent. alcoholic potash (250 c.c.) and left for 2 days. 2 *N* potash (100 c.c.) was added later and the alkaline solution extracted with ether several times. The ethereal solution gave after drying and distilling the solvent an oil (nearly 2 g.). From 10 g. of this oil a fraction (2 g.) boiling at 145-147°/24 mm. was obtained. This has not been further investigated, but was found to contain C, 67.8; H, 7.5 per cent. The flask contained after distilling all oily products till 180° a thick residue which set to a semi-solid mass on cooling. On filtering the crystalline solid and purifying it from alcohol it melted at 176° (sublimes in vacuum at 100°) and was identified with the bridged *cyclo*hexenone (Found: C, 78.4; H, 8.5; *M*, 245. $C_8H_{10}O$ requires C, 78.2; H, 8.2 per cent. *M*, 122).

Hydrolysis of ester (II) *with* (*b*) 60 *per cent. sulphuric acid*.—The ester (100 g.) was mixed with 60 per cent. sulphuric acid (100 c.c.) and kept aside for 2 days. After diluting with water, it was made alkaline and extracted with ether; this gave an oil as before (2.5 g.) which on being treated as above gave the ketone m.p. 176°. Yield (1 g.)

Dehydration of dimethyldihydroresorcinol (V) *with phosphorus pentoxide*: *Formation of the bridged* cylo*hexenone* (IV).—Dimethyldihydroresorcinol (12 g.) was dissolved in anhydrous xylene (200 c.c.) and phosphorus pentoxide (12 g.) added. It was warmed on the water bath for 8 hours and then heated in an oil bath at 120° for a couple of hours. Xylene was distilled and the brown residue decomposed with water and extracted with ether. The ethereal solution after being washed with 2 *N* sodium hydroxide gave a resinous mass (o. 8 g.) from which was obtained only a trace of IV after repeated crystallisation from benzene alcohol mixture.

Ethyl cyclo*hexane*-1 : 1 : 2-*trimethyl*-3 : 5-*diketo*-6-*carboxylate* (VII).—To a solution of sodium (12 g.) in alcohol (150 c.c.) were added methyl acetoacetic ester (72 g.) and *iso*propylidene malonic ester (100 g.) and the mixture boiled under reflux for 12 hours. Alcohol was distilled and the residue on acidification evolved carbon dioxide. The ether extract gave an oil (122 g.) from which was collected a fraction distilling at 187°/21 mm., yield (35 g.); d^{23° 1.3151, $n_d^{23^\circ}$ 1.4838, $[R_L]_D$ 56.99 (calc.: 57.09) (Found: C, 63.4; H, 9.3; *M*, 232. $C_{12}H_{18}O_4$ requires C, 63.7; H, 8.0 per cent. *M*, 226).

The *disemicarbazone* melted at 225° (decomp.) (Found: N, 14.6. $C_{13}H_{21}O_4N_3$ requires N, 14.8 per cent.).

Condensation of methyl mesityl oxide with malonic ester. Synthesis of ester (VII).—Methyl mesityl oxide prepared by Blaise-Maire reaction on Perkin and Thorpe's trimethyl acrylyl chloride (*J.C.S.*, 1896, **69**, 1080), b.p. 142-144°/684 mm. was condensed according to Bardhan (*J.C.S.*, 1928, 2608) with malonic ester. The oil obtained from the reaction product distilled at the same temperature as the previously mentioned ester (X), and had the same constants.

Hydrolysis of ester (VII) to 1 : 1 : *2-trimethyl-3 : 5-diketo*cyclo*hexane,* (XIII).—Ester (VII) (10 g.) was mixed with concentrated baryta (150 c.c.) and boiled under reflux for 12 hours. The solution was evaporated to dryness, acidified with concentrated hydrochloric acid and extracted with ether, when it gave a thick brown liquid (2.5 g.) which solidified in a vacuum desiccator. The solid crystallised from dilute alcohol in needles, m.p. 100° (Found: C, 69.8; H, 9.6. $C_9H_{14}O_2$ requires C, 70.1; H, 9.2 per cent.) and was identified with Crossley's (*J. C. S.*, 1901, **79**, 138) trimethyldihydroresorcinol obtained by hydrolysing the condensation product of mesityl oxide with methyl malonic ester. Alcoholic potash was found to be a better hydrolysing agent.

Dehydration of (VII). *Formation of the bridged dehydrated ester* (VIII).—A mixture of trimethyldihydroresorcinol ester (VII) (20 g.) and 20 per cent. alcoholic potash (30 c.c.) was kept at room temperature for 48 hours. The alcohol-free reaction mixture was neutralised and ether extracted and washed with sodium carbonate to remove all the resorcinols and other soluble products, yield (12 g.). The main product distilled at 145-147°/20 mm., yield (4 g.). d^{24} 1.0582, $n_d^{24°}$ 1.4830, $[R_L]_D$ 56.18 (calc.: 54.41) (Found: C,69.0; H, 7.9; *M*, 205. $C_{12}H_{16}O_3$ requires C, 69.2; H, 7.7 per cent.; *M*, 208). The *semicarbazone* had m.p 205° (decomp.) (Found: N, 15.5. $C_{13}H_{19}O_3N_3$ requires N, 15.1 per cent.).

50 per cent. sulphuric acid also effects dehydration.

Hydrolysis of the bridged ester (VIII) *to the resorcinol* (XIII).—The ester (1 g.) was boiled under reflux with concentrated baryta (25 c.c.) The solution was evaporated to small bulk, acidified with hydrochloric acid, extracted with ether and the solid obtained therefrom crystallised from dilute alcohol, m.p. 100°.

Formation of the bridged ester (III) *from Scheiber and Miesel's ester* (I).—A mixture of Scheiber and Miesel's ester (40 g.) and 20 per cent. alcoholic potash (25 c.c.) was kept at room temperature for 2 days. Similarly another portion of the ester (40 g.) was kept with 50 per cent. sulphuric acid (25 c.c.) for the same period. Afterwards the products were worked up in the same way the yield being (12 g.) and (15 g.) respectively. It distilled as a colourless oil at 195°/20 mm.; $d^{23.5°}$ 1.1386, $n_d^{23.2°}$ 1.4812, $[R_L]_D$ 66.49 (calc.: 65.30) (Found: C, 62.9; H, 8.3; *M*, 272. $C_{14}H_{18}O_5$ requires C, 63.2; H, 8 per cent.; M, 266). The *semicarbazone* had m.p. 232° (decomp.) (Found: N, 12.8. $C_{15}H_{21}O_5N_3$ requires N, 13.0 per cent.).

Hydrolysis of the ester (III) *to the bridged cyclohexenone* (IV).—A mixture of the ester (5 g.) and 60 per cent. sulphuric acid (20 c.c.) was warmed on the water bath for 4 hours. The resulting solution after dilution was extracted with ether from which a solid m.p. 176° (2 g.) was obtained, identified with the bridged *cyclo*hexenone.

Hydrolysis of ester (VII) *to the bridged* cyclo*hexenone* (X).—The ester (XI) (20 g.) was mixed with 20 per cent. alcoholic potash (40 c.c.) and kept aside for nearly a week. The same quantity of the ester was separately warmed on the water bath for 4 hours with 60 per cent. sulphuric acid (50 c.c.). The ether extract after sodium carbonate treatment gave a light yellow pleasant smelling liquid (5 g. and 6 g. respectively) distilling at 105/20 mm. d^{25} 0.9508, $n_d^{25°}$ 1.4743, $[R_L]_D$ 40.24 (calc.: 38.91) (Found: C, 79.0; H, 9.3; *M*, 142. $C_9H_{12}O$ requires C, 79.4; H, 8.8 per cent.; *M*, 136).

The *semicarbazone* crystallised from alcohol in flaky needles, m.p. 186° (Found: N, 21.5. $C_{10}H_{15}ON_3$ requires N, 21.7 per cent.).

Conversion of (X) *to the dihydroresorcinol* (XVII).—The bridged *cyclo*hexenone (2 g.) was boiled under reflux with 10 per cent. alcoholic potash (20 c.c.) for 5 hours and the alcohol distilled. The ether extract gave a semi-solid crystallising from dilute alcohol, m.p. 100°; identified with Crossley's trimethyldihydroresorcinol (*loc. cit.*).

*Methylation of Vorländer's ester to ethyl*cyclo*hexane*-1 : 1 : 4-*trimethyl*-3 : 5-*diketo*-6-*carboxylate* (XIV).—Vorländer's ester (106 g.) was slowly added to a solution of sodium (12 g.) in alcohol (150 c.c.) and the mixture boiled under reflux for 12 hours with methyl iodide (75 g.). The alcohol-free residue on acidification and extraction with ether gave a semi-solid. The oily products were filtered, and the solid

crystallised from a mixture of ethyl acetate-petroleum ether in fine clusters of needles, m.p. 108°, yield (70 g.) (Found: C, 63.3; H, 8.5; *M*, 218. $C_{12}H_{18}O_4$ requires C, 63.7; H, 8 per cent.; *M*, 226.)

The *disemicarbazone* crystallised from alcohol; m.p. 236° (decomp.) (Found: N, 14.5. $C_{13}H_{21}O_4N_3$ requires N, 14.8 per cent.).

Formation of (XIV) *from Scheiber and Miesel's ester* (I).—Scheiber and Miesel's ester (140 g.) was added to a solution of sodium (12 g.) in alcohol (150 c.c.) to which was added methyl iodide (75 g.) and the mixture boiled under reflux for 12 hours. After the usual process of purification a crude product (200 g.) was obtained which on distilling under diminished pressure gave a thick oil (10 g.) distilling at 165°/5 mm. and solidifying on cooling. It melted at 106°. The residue charred on distillation.

Hydrolysis of (XIV) *to the resorcinol* (XVII).—The ester (10 g.) was mixed with 20 per cent. alcoholic potash (50 c.c.) and kept for a week. After removing alcohol and acidifying, the ether extract gave a solid (5 g.) which crystallised from benzene-petroleum ether as rhombic needles m.p. 161°, identified with Desai's (*loc. cit.*) methylated dimethyldihydroresorcinol (Found: C, 69.9; H. 10.2. $C_9H_{14}O_2$ requires C, 70.1; H, 9.9 per cent.).

Action of 60 *per cent. sulphuric acid on the ester* (XVIII). *Formation of the lactone* (XV) *or* (XVI).—To the ester (50 g.) was added 60 per cent. sulphuric acid (100 c.c.) when it became warm; the mixture was kept at room temperature for 3 days. Heating on the water bath finished the reaction sooner without any loss in yield. After dilution the mixture was ether extracted and washed with sodium carbonate to remove all the resorcinols. The ethereal solution gave a thick oil (6 g.) which in a vacuum desiccator at low temperature sets to a crystalline mass. This freed from the oily impurities, crystallised from alcohol as beautiful cubical plates, m.p. 79° (Found: C, 63.4; H. 7.4; *M*, 190. $C_{10}H_{12}O_3$, $\frac{1}{2}H_2O$ requires C, 63.5; H, 6.9 per cent.; *M*, 189). The product was dehydrated by keeping over phosphorus pentoxide at 100° (Found: C, 66.1; H, 7.1. $C_{10}H_{12}O_3$ requires C, 66.6; H, 6.7 per cent.). It was neutral to sodium carbonate or cold alkali. With hot alkali it titrated as a dibasic acid, and was estimated by back titration. (Equiv. 100; Calc. 95).

The *semicarbazone* crystallised from alcohol in plates m.p. 188° (decomp.) (Found: N, 17.5. $C_{11}H_{15}O_3N_3$ requires N, 17.7 per cent.).

Hydrolysis of the lactone (XV) *or* (XVI) *to the resorcinol* (XVII). —The lactone (2 g.) was boiled under reflux with 10 per cent. alcoholic

potash (25 c.c.) for 4 hours. After the usual ether extraction it gave a thick oil (1 g.) which solidified and was crystallised from benzene-petroleum ether, m.p. 161°; identified with Desai's (*loc. cit.*) methylated dimethyldihydroresorcinol (Equiv. 156; Calc. : 154).

The author has great pleasure in acknowledging his gratitude to Dr. P. C. Guha, D.Sc., and Sir Martin Forster, F.R.S., for their continued interest and helpful suggestions during the progress of this investigation.

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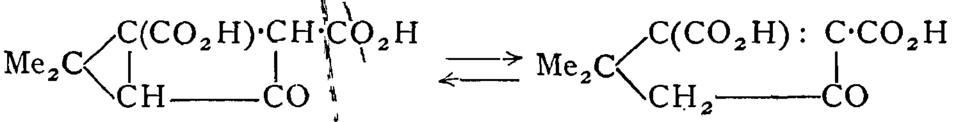
STUDIES IN BRIDGE FORMATION

PART IV. TAUTOMERISM IN *iso*PHORONE

By P. S. Mayuranathan.

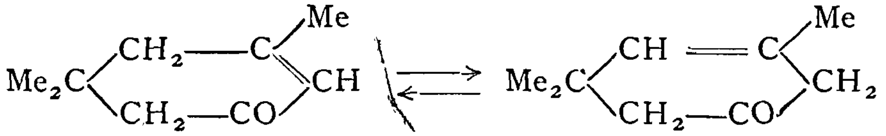
In their opening paper on *intra-annular tautomerism* (*J. C. S.*, 1922, **121**, 128) Farmer, Ingold, and Thorpe have shown that dimethyldi*cyclo*pentanonedicarboxylic acid which by synthesis would possess structure (I) behaves also as though it had the unsaturated structure (II).



I II

An attempt is made in this paper to trace a similar phenomenon in the molecule of *iso*phorone (III).

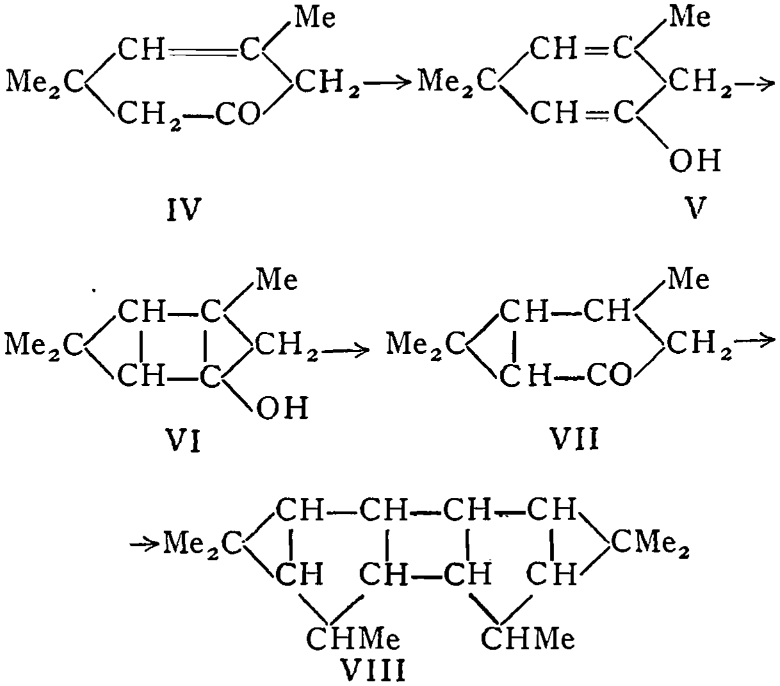
Two isomeric forms of *iso*phorone (III) and (IV) have been discovered by Bredt and Rubel (*Annalen*, 1898, **299**, 160), and Kerp and Muller (*ibid.*, 1898, **299**, 193) depending on the shift of a double bond in the molecule.



III IV

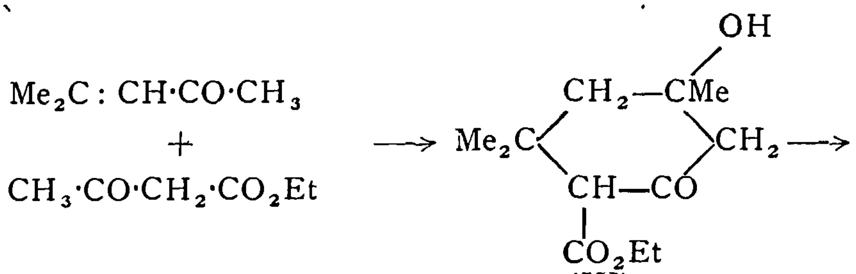
Lately, Baker (*J.C.S.*, 1926, 626) has ingeniously applied the principle of intra-annular tautomerism to the molecule of *iso*phorone and in accordance with the scheme outlined below claims to have obtained, on reducing the ketone by Clemmensen's method, a hydrocarbon, $C_{18}H_{28}$ (VIII), m.p. 112°, possessing two *cyclo*propane bridged bonds. Proof for the constitution of (VIII) was that on oxidation with alkaline

permanganate it gave caronic acid. On attempting to reproduce these reactions the author was unable to obtain the solid hydrocarbon (VIII), the final product being a liquid with the formula $C_{18}H_{32}$. All attempts at its oxidation to caronic acid proved ineffective showing that the *cyclo*propane bridged bonds in (VIII) have been saturated; the possibility of the *cyclo*butane ring opening up is also unlikely because of the consequent formation of a ten-membered ring.

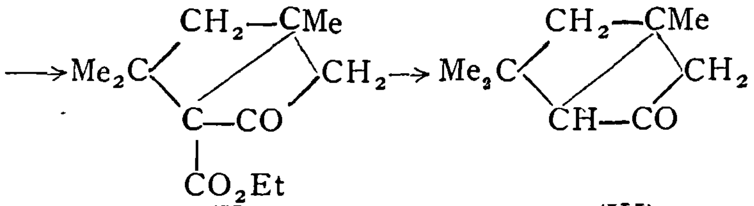


IV V VI VII VIII

Of the two known syntheses of *iso*phorone one is due to Bredt and Rubel (*loc. cit.*) by condensation of three molecules of acetone, and the other to Knoevenagel and Fischer (*Annalen*, 1897, **297**, 185) from mesityl oxide and acetoacetic ester. The latter synthesis is analogous to that of the bridged *cyclo*hexenones (Part II and III) and on *a priori* considerations it appeared possible that during formation (the dehydration perhaps enhanced by the actual presence of a hydroxyl *para* to the reactive hydrogen atom) it should have passed through a bridged phase (XI). This demands less strain on the molecule than the double bridged phase (VI) proposed by Baker (*loc. cit.*). The mechanism of the formation of *iso*phorone can be represented by the following scheme :



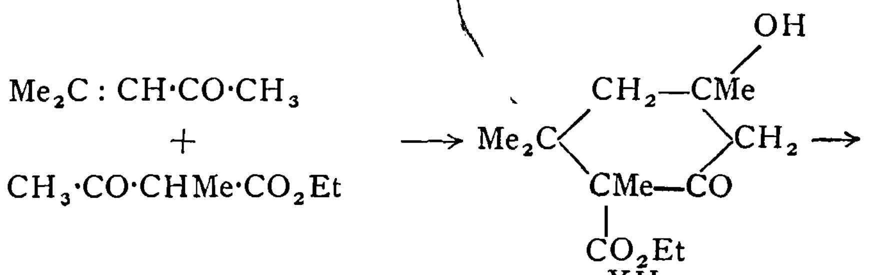
(IX)



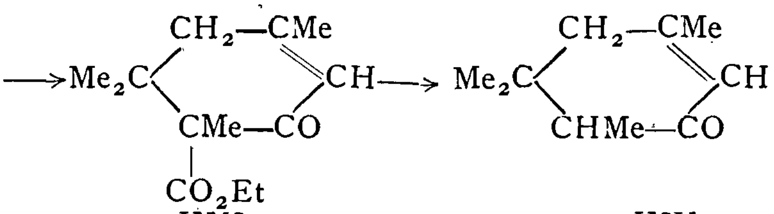
(X) (XI)

The hydroxy ester (IX) as soon as it is formed gets dehydrated by sodium ethoxide to the bridged ester (X) accounting for the non-isolation of the former and the alcoholic sodium hydroxide incidentally formed hydrolyses ester (X) to the ketone (XI). The constitution of the dehydrated ester is ambiguous as it can have either a bridged or a double bonded form according as dehydration takes place across or along the ring, but if preference be given to dehydration involving the reactive methylene group, the resulting ketone should have a bridged structure. The dehydrated ester, however, on methylation gives (XIII), probably due to reactivity acquired through the wandering of a hydrogen atom.

The main product obtained by condensing mesityl oxide with methyl acetoacetic ester is the ester (XII) : this contrast with the foregoing experiment establishes that a decisive factor in dehydration is the hydrogen atom *para* to the hydroxyl rather than the less reactive one attached to the ketomethylene group.



XII



XIII XIV

EXPERIMENTAL

Clemmensen reduction of iso*phorone to the hydrocarbon* $C_{18}H_{32}$.—To a mixture of *iso*phorone (60 g.) and amalgamated zinc (300 g.) in a litre flask was gradually added concentrated hydrochloric acid (540 c.c.) and the mixture boiled under reflux slowly for 6 hours until all the zinc had reacted. The diluted solution on ether extraction gave a viscous oil (52 g.) which on steam distillation gave no solid hydrocarbon. On repeating the experiment several times a camphoraceous oil (4 g.) was collected which distilled at 102°/5 mm. The original oil on distillation gave the same hydrocarbon, yield 5 per cent. (Found: C, 86.5; H, 13.2; *M*, 246. $C_{18}H_{32}$ requires C, 87.1; H, 12.9 per cent.; *M*, 248).

Formation of iso*phorone ester* (X) *and* iso*phorone* (III).—A mixture of mesityl oxide (100 g.) and acetoacetic ester (133 g.) was added gradually under ice-cooling to a solution of sodium (23 g.) in alcohol (300 c.c.) and kept in the ice-chest for 10 days and then boiled under reflux for 4 hours, when carbon dioxide was profusely evolved. The alcohol-free residue was acidified and extracted with ether when a colourless oil (III) was obtained, b.p. 135°/20 mm., yield 75 per cent., $d^{24°}$ 0·9461, $n_D^{24°}$ 1·4752, $[R_L]_D$ 41·10 (calc.: 41.11) (Found: C, 78.0; H, 14.2. $C_9H_{14}O$ requires C, 78.3; H. 14.0 per cent.). The *semicarbazone* had m.p. 196°.

When refluxing was omitted and the mixture neutralised by passing carbon dioxide, an appreciable quantity (yield, 5 per cent.) of colourless oil distilled at 162°/20 mm. (X), d25° 1·065, $n_D^{25°}$ 1·4788 $[R_L]_D$ 55.91 (calc. 54.88) (Found: C, 68.5; H, 8.9; *M*, 210. $C_{12}H_{18}O_3$ requires C, 68.6; H. 8.6 per cent.; *M*, 210). The *semicarbazone* had m.p. 218° (decomp.) (Found: N, 15.7. $C_{13}H_{21}O_3N_3$ requires N, 15.7 per cent.).

Methylation of ester (X) *to* (XIII).—To sodium (2.3 g.) in alcohol (30 c.c.) were added ester (X) (20 g.) and methyl iodide (15 g.) and the contents boiled under reflux for 4 hours. The resulting ester distilled at 175°/20 mm., and was identified with (XIII).

Formation of ketone (XIV) *and esters* (XIII) *and* (XII).—A mixture of mesityl oxide (100 g.) and methyl acetoacetic ester (147 g.) was added to sodium (23 g.) dissolved in alcohol (300 c.c.) and kept in the ice-chest for 10 days. It was found necessary to reflux the mixture for 6 hours to complete the reaction. On working up the product and distilling, three fractions were collected; fraction (i) was a light yellow oil (15 g.) b.p. 143°/20 mm., $d^{23.5°}$ 09616, $n_D^{23.5°}$ 1·4900, $[R_L]_D$ 45·69 (calc.: 45.74) (Found: C, 78.8; H, 10.8. $C_{10}H_{16}O$ requires C, 78.9; H, 10.5 per cent.). The *semicarbazone* melted at 200° (Found: N, 19.4. $C_{11}H_{19}ON_3$ requires N, 20 per cent.); fraction (ii) (20 g.) distilled at 175°/20 mm., $d^{24°}$ 1·1151, $n_D^{24°}$ 1·5205, $[R_L]_D$ 61·16 (calc.: 61.23) (Found: C, 69.5; H, 9.2. $C_{13}H_{20}O_3$ (XIII) requires C, 69.7; H, 8.9 per cent.). The *semicarbazone* had m.p. 235° (decomp.) (Found: N, 14.5. $C_{14}H_{23}O_3N_3$ requires N, 14.9 per cent.). Fraction (iii) (XII) (136 g.) distilled at 196°/20 mm. as a thick yellow oil (Found: C, 64.2; H, 9.4. $C_{13}H_{22}O_4$ requires C, 64.4; H, 9.1 per cent.).

In conclusion the author wishes to express his grateful thanks to Dr. P. C. Guha, D.Sc., and Sir Martin Forster, F.R.S., for their kind interest and helpful suggestions in this work.

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SYNTHESIS OF PROTEINS IN PLANTS. PART I. CONVERSION OF NITRATES INTO PROTEIN IN *HELIANTHUS ANNUS*, LINN.

By K. S. Varadachar.

Although the study of synthesis of proteins in plants has drawn the attention of a large number of workers, yet very little definite information is available about this important branch of plant physiology. This is chiefly due to want of adequate technique for controlling the intake of nitrogen by plants as also to lack of accurate methods for estimating small quantities of nitrogenous substances formed at various stages in the plant.

A number of useful observations have been recorded by individual workers but the uncertainty regarding the first product of photosynthesis has invariably led to a good deal of confusion in interpreting these observations. At various times hydrocyanic acid, ammonia or potassium nitrite has been mentioned as reacting with formaldehyde and thence the mechanism of protein-synthesis elaborated. The fact that protein-synthesis is not dependent on light but can proceed even in the dark has been a great drawback in these explanations. Several theories have also been postulated on the basis of observations made under *in vitro* conditions but most of them have only academic interest.

The theories regarding the mechanism of protein-synthesis can be broadly divided into three groups—(1) those that assume the preliminary formation of the several amino acids and their subsequent condensation to proteins, (Trier, G. Einfachen-flanzenbasen und ihre Beziehungen zum Aufbau der Eiweisstoff und Lecithin, Berlin, 1912; Baudisch u. Meyer, *Central Bakt;* Abt. II. 1912, **45**, 1771; Baudisch u. Klinger, *Ibid.*, 1916, **49**, 1167; Lob, *Zeit. f. Elektrochem.*, 1906, **12**, 282), (2) those that assume direct interaction between the sugars formed during photosynthesis and potassium nitrite or ammonia arising out of the decomposition of absorbed nitrate, resulting in the synthesis of protein in plant (Baly *et al.*, *J.C.S.*, 1922, **121**, 1078; Huppert, Konstitution und Konifiguration der Eiweisstoff, Leipzig, 1925; Loew, *Biochem. Z.*, 1912, **41**, 224; *Chem. Ztg.*, 1912, No. 7 and *Ber.*, 1913, **46**, 684), and (3) those that assume a preliminary formation of amides, especially asparagine and their subsequent transformation into proteins (Muller, *Landwirtschaft Versuchs-St.*, 1886, **33**, 311; Nakamura, *Bull.*

Agric. Coll. Tokyo, 1897, 465; Saposchnikow, *Bot. Zent.*, 1895, **63**, 246; Prinanischnikoff, *Russ. J. Expt. landwirt,* 1912, **13**, 5; *Rev. gen. Bot.* 1913, **25**, 5).

The object of the present investigation is to throw some fresh light on this important problem by directly feeding the plant with various nitrogenous constituents and following the attendant biochemical changes. The present paper relates to the changes undergone by nitrates in the sunflower plant (*Helianthus annus,* Linn.).

EXPERIMENTAL.

The experiments were conducted with sand-cultured plants in glazed earthenware pots. The sand was repeatedly washed with water and, after drying, filled in at the rate of 60 lbs. per pot. It was then mixed with lime at 50 g. per pot and left to weather for about 4-5 weeks, after which potassium acid phosphate (4.8 g.) and potassium sulphate (2.7 g.) were added.

The sunflower plants (Sutton's Giant Yellow variety) were raised on manured beds until the stems attained a thickness of about 8 mm. and then transplanted into the pots. The potted plants were watered at the rate of 2 litres for every alternate day.

After about 15 days when the plants had established themselves, they were injected with distilled water and their ability to take in aqueous nutrients tested from day to day. The technique of injection developed after numerous trials and adopted for this investigation was as follows :—

A glass tube about 1 cm. in diameter and 10 cms. long was fused to another one of diameter 3 mm. The smaller tube was bent twice at right angles. To the end of the smaller tube was attached one inch of rubber tubing and to this was fixed an 'Esco' record syringe needle of stainless steel. To inject a plant, the needle was inserted slantwise into the stem as near the soil as possible so that it was well fixed in the plant. It was then removed and the bore cleaned with a brass wire and reinserted. The point at which the injection was made was protected with a little beeswax. Distilled water was placed in the side tube which acted as a reservoir and observed for a couple of days to ensure that the uptake was continuous and that there was no choking in the needle. When required, the distilled water was pipetted out and the experimental solution introduced in known quantities. The

tube was held in position by means of a zinc clip attached to an iron rod and a test tube inverted over the reservoir to minimise evaporation.

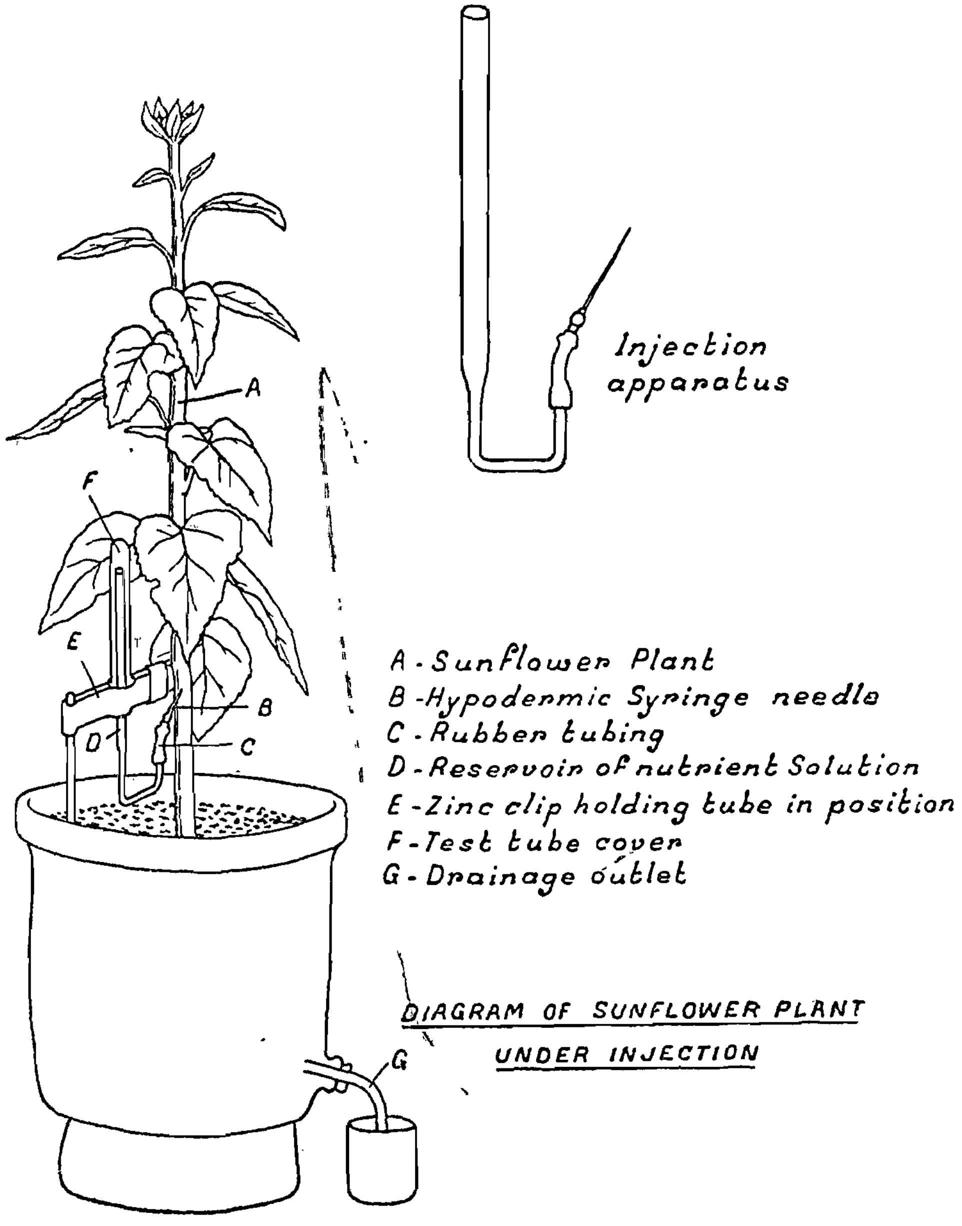

DIAGRAM OF SUNFLOWER PLANT UNDER INJECTION

It was observed that almost all the plants took in water quite readily and continued to grow healthy though the leaves soon began to turn yellow owing to lack of nitrogen.

After ensuring that the passage of water into the plant was steady and continuous, the distilled water was pipetted out and known quantities of standard nitrate solution (2.5 per cent.) placed in each of the reservoir tubes. Several plants were left with water only to serve as controls.

The above treatment with nitrate solution was continued for about a week. Specimens of whole plants were collected at intervals, biological activity stopped by heating for 4 hours at 100° and drying completed at 60°. The dried specimens were analysed for total weight, total nitrogen including nitrates, humin N, amide N, basic N, and non-basic N. The quantity of nitrate absorbed by each plant at the time of observation was also determined by the difference between the quantities introduced at the beginning and that left unabsorbed when sampling.

A preliminary trial was conducted in July 1930 and subsequently repeated in greater detail in February 1931 and 1932 respectively. It was observed that the plants recovered their normal colour within 48 hours and remained healthy throughout the experiment. The results show that the injection method of feeding plants is a highly efficient form of introducing large quantities of nutrients into plants without any detrimental effect.

The total nitrogen was found according to the Arnold-Gunning modification of the Kjeldahl method. Considerable difficulty was experienced in selecting suitable methods for estimating small quantities of the different forms of nitrogen in plant material, owing to the amount of material available for analysis being small. Thus the estimation of nitrates presented great difficulty and several standard methods were tried and found unreliable. The most consistent results were obtained by repeated extraction with hot water followed by reduction with Devarda's alloy.

In regard to the distribution of nitrogen in the plant, the method of differentiating it in terms of solubility in various solvents like water, alcohol and sodium chloride would not lead to any conclusive figures. Van Slyke's method of analysis is not applicable to a material like dried plant. In view of the above the micro method of determining Hausmann numbers developed by Thiemann (*Biochem. J.*, 1926, **20,** 1190) was employed. The material was dropped into boiling 20 per cent. hydrochloric acid and hydrolysed for 36 hours. The hydrolysate was then separated into humin, amide, basic and non-basic fractions. The nitrate content of each sample was also determined.

A large mass of useful data were collected during the three seasons in which the experiments were conducted but with a view to facilitating discussion only the more representative figures have been given in the following tables :—

TABLE I.

Effect of nitrate injection on the dry weights of plants.

No. of days under injection	Treated Plants		Control Plants	Percentage increase in dry weight over controls
	Nitrate absorbed in mg.	Dry weight in gms.	Dry weight in gms.	
1	8·5	11·78	10·8	+10·0
2	14·9	7·51	6·42	+16·9
3	6·4	6·00	7·73	−23·0
4	7·9	11·21	11·85	− 5·4
5	11·1	11·27	9·09	+23·8
6	16·9	9·66	6·60	+46·3
7	13·2	13·28	8·40	+58·0
8	4·1	11·02	9·69	+13·4
11	3·6	12·45	6·69	+86·4
12	7·3	10·11	7·05	+43·4
13	5·7	8·27	9·91	−17·0
14	6·5	10·61	7·51	+41·3
15	9·8	12·65	7·44	+70·0
17	?	10·82	7·71	+40·2
18	8·1	12·18	9·79	+24·5
19	10·4	11·63	8·44	+37·9
20	?	17·63	11·24	+57·0
21	11·5	13·02	9·59	+35·5
22	12·6	13·71	11 20	+22·3
23	9·4	9·58	9·14	+ 5·8
24	.0·2	12·25	7·87	+55·5
25	9·9	13·82	10·47	+32·0
26	11·7	16·39	12·74	+28·7
27	5·9	9·59	9·65	− 0·7
28	9·7	11·74	10·27	+14·3

Average increase in dry weight = 25·6 per cent.

TABLE II.

Season—1931.

Number of days under injection	Total nitrogen in plant	Nitrate nitrogen in plant
	mgms.	mgms.
2	67·1	10·7
4	92·5	4·2
5	112·6	10·6
7	105·4	13·2
11	205·2	26·4
12	126·9	1·9
15	122·6	7·2
18	99·5	2·3
20	233·2	19·2
21	101·5	5·5

TABLE III (a).

Season—1930.

Date of commencement of injection, 25-8-1930.

Number of days under injection	Total nitrogen per cent.	Humin nitrogen per cent.	Percentage of humin N. in total N.
4	0·79	0·33	41·0
9	0·94	0·40	43·2
15	0·83	0·34	41·0

TABLE III (b).

Season—1931.

Number of days under injection	Percentages on dry weight			
	Humin N.	Amide N.	Diamino N.	Non-basic N.
2	0·11	0·10	0·15	0·40
4	0·11	0·06	0·10	0·53
5	0·12	0·06	0·10	0·67
6	0·14	?	0·13	0·61
7	0·12	0·05	0·13	0·39
8	0·11	0·01	0·13	0·50
11	0·12	0·15	0·11	1·77
12	0·12	0·08	?	0·91
13	0·13	0·05	0·13	0·45
15	0·14	0·05	0·09	0·67
17	0·13	0·04	0·14	0·42
19	0·12	?	0·11	0·57
20	0·16	0·05	0·09	0·51
22	0·11	0·09	?	0·65
23	0·13	0·08	0·16	0·45
25	0·12	0·06	?	0·47
26	0·13	0·07	0·14	0·88
27	0·14	0·08	0·12	1·77

The effect of nitrate injection on the dry weights of plants was determined as follows: pairs of plants which were very nearly alike with regard to height, girth and general development at the time of the commencement of the experiment were taken out at stated intervals and their respective dry weights compared. Since the specimens were independent plants and had absorbed varying amounts of nitrate, the quantitative effect of the treatment with time could not be evaluated. The plants were therefore treated as representatives of two sets of population, one subjected to injection and the other left untreated and the effects on corresponding specimens compared. The results (Table I) show that the treatment has been beneficial and that the majority of

plants have shown distinct increase in dry weight as the result of nitrate injection. The above observation being perhaps the first record of plant growth being favoured by direct feeding with a mineral nutrient, the experiments were repeated carefully during successive seasons and the results confirmed.

The absorption of nitrate (as shown in column 2, table I) being a mechanical process dependent on such variable factors as temperature, humidity, transpiration etc. does not show any regularity. The results show, all the same, that the plants have absorbed useful amounts of nitrogen and have grown normally without, in any way, being affected by either the injection or the high concentration of the nutrient fed into it.

From column 3 of table II, it might be seen that there is increase in the nitrate content upto the 11th day followed by during successive days. There is however no regularity about this accumulation nor does the quantity bear any proportion to the total nitrogen in the plant thereby suggesting that there is continuous transformation of nitrate into other forms of nitrogen.

The large difference with regard to the humin nitrogen obtained during the 1930 and 1931 series is quite striking. In the former, the humin [table III (a), column 3] accounted for about 40 per cent. of the total nitrogen while in the latter [table III (b), column 2] it was less than a third of that quantity. The difference between the two sets of figures is quite inexplicable particularly when considering that the hydrolyses were conducted under identical conditions. Such high figures as those obtained in the first season were not repeated at any later stage but even those of the latter ones should be regarded as excessive so that the accuracy of the distribution of nitrogen has to a large extent been affected by the precipitation of quite considerable quantities in the form of the comparatively unknown entity humin.

The variation observed with regard to the figures for amides [column 3, table III (b)] suggest the formation of those compounds as intermediary products, in the building up of proteins. This supports one of the existing theories (*loc. cit.*) regarding the mechanism of protein synthesis. Whether this is due to the formation of asparagine or any other amide, it is not possible to say at this stage of the investigation. It is hoped that later research will throw more light on this aspect of the problem.

The figures for diamino nitrogen [table III (b), column 4] representing the combined values for arginine, histidine, lysine and cystine

fractions do not lead to any conclusion. Fractionation of the constituents by micro methods is now in progress and will form the subject of a later communication.

The figures for non-basic nitrogen (column 5) show a correlation with those for amide. The rise and fall correspond inversely to similar alteration in the amide value showing a relation between those two classes of substances. Thus, from table III (b), it is clear that the transformation of nitrate in the plant is accompanied by periodic accumulation and disappearance of amides and corresponding reverse changes in the non-basic fractions.

With a view to shortening the interval between the observations the whole series of experiments were repeated in 1932 sampling the plants day and night at intervals of two hours. The first sample was taken 2 hours after the injection of nitrate and the last 50 hours later. The figures for the analysis of the various samples collected at six-hour intervals are given in table IV.

TABLE IV.

Time of sampling hrs.	Total dry weight of plant in gms.	Total N in whole sample in mg.	Nitrate N in whole sample in mg.	Humin N	Amid N	Diamino N	Non-basic N
				per cent.			
0	14·62	118·2	0·6	0·16	0·13	0·16	0·41
0	14·08	124·9	2·8	0·16	0·12	0·14	0·32
6	19·05	144·0	0·8	0·15	0·13	0·13	0·35
12	16·05	202·7	1·3	0·19	0·12	0·16	0·79
18	18·94	...	3·7	0·12	0·09	0·12	0·52
24	17·66	...	2·1	0·17	0·16	...	0·66
30	17·24	159·1	0·7	0·16	0·11	0·17	0·48
36	16·20	372·6	4·5	0·30	0·19	0·37	1·40
42	37·65	349·7	1·5	0·14	0·09	0·13	0·57

It may be seen from the above that the nitrate content in the plant remains comparatively small throughout the period of observation. The total nitrogen does however show a tendency to increase, thereby indicating that conversion of the nitrogen into other forms takes place at a very rapid rate so that nitrate content is not high at any stage during the experiment.

The amide nitrogen under observation shows the same type of rise and fall as that observed before thereby indicating that the nitrate passes through the amide stage. But there is no correlation between the amide content of the plant and the figures for non-basic nitrogen as was noted before.

Although the foregoing observations have not revealed any striking feature in the mechanism of synthesis of proteins in plants they have yet shown that the technique of direct feeding offers immense possibilities for future study. Further work is therefore in progress with a view to refining the technique and standardising the conditions for estimation of the different forms of nitrogen.

SUMMARY.

A direct method of feeding of plants with nutrients is described. This technique has been applied to the study of protein synthesis by feeding nitrogen-starved sunflower plants with potassium nitrate solution and studying the attendant changes.

There is evidence to suggest that there is continuous conversion of nitrate into other forms of nitrogen. The added nitrogen would appear to pass through the amide stage before being converted into protein.

In conclusion the author wishes to express his thanks to Dr. V. Subrahmanyan, D.Sc., F.I.C., for suggestions and criticism.

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CONTRIBUTIONS TO THE STUDY OF SPIKE-DISEASE OF SANDAL (*SANTALUM ALBUM*, LINN.)

Part XV. The role of plant acids in health and disease.

By A. V. VARADARAJA IYENGAR.

It was shown in an earlier communication (*J. Indian Inst. Sci.*, 1928, **11A**, 103) that the hydrogen-ion concentration of the tissue fluid of spiked sandal was higher than that of the healthy tree. The reaction of the fluid from a partially diseased plant is, at the outset, more alkaline than that of the healthy one, but became more acid with the progress of the disease (*Ibid.*, 1930, **13A**, 295). Since the H-ion concentration of plant juice is believed to be largely due to the presence of organic acids, the present investigation was undertaken with a view to determining the nature and distribution of those acids in healthy and diseased sandal.

Materials and Methods.—The present study was carried out on leaf material derived from a number of forest areas, particularly Ragihalli and Uttarahalli in Bangalore District and Nognoor and Denkanikota in North Salem.

The leaves were minced and extracted with dilute (2.5 per cent.) hydrochloric acid. The extract was neutralised, concentrated to a small volume and treated with two volumes of 95 per cent. alcohol. After removing the precipitate, the filtrate was treated with hot saturated lead acetate. The mixture of lead salts thus obtained was suspended in water and freed from adsorbed sugars by repeated passage of carbon dioxide, washed carefully and dried. It was then treated with either dilute sulphuric acid or hydrogen sulphide to liberate the free acids. The filtrates were examined for the presence of oxalic, malic, citric, succinic and tartaric acids according to the method of Schmalfuss and Keitel (*Z. physiol. Chem.*, 1924, **138**, 156). The method was first tested by working with small quantities of known acids both individually and in mixtures. It is possible to estimate the quantities of acids by comparison of colour intensities, but in the present case, no quantitative studies were carried out by this method owing to the small amounts of acids available.

In another set of experiments, the final solution containing the free acids was extracted with ether in an apparatus which was first

devised in this laboratory and since independently described by Quick (*Ind. Eng. Chem. Analyt. Edn.*, 1933, **5**, 76). The ether soluble acids were identified in the manner described above.

In a further set of trials the residue after extraction with ether was subjected to micro-sublimation and the sublimates obtained at various temperatures identified as their characteristic salts.

The results obtained by the three methods agreed closely with each other, and may be summarised as follows :—

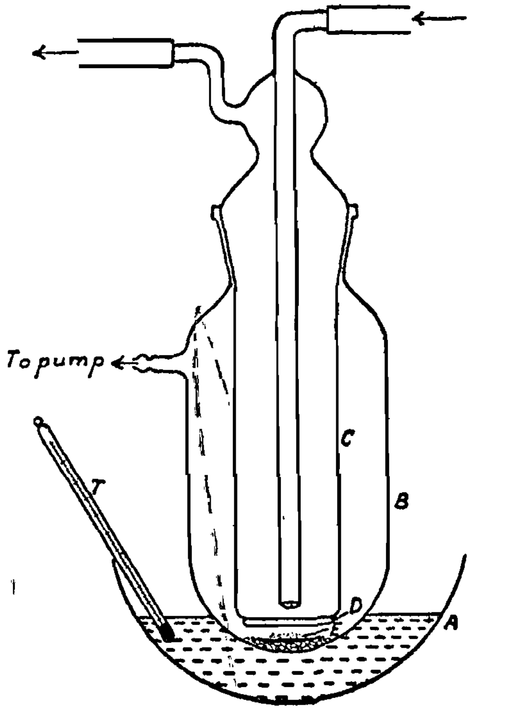
The three hydroxy acids, malic, citric and tartaric increased in quantity (from ++ in the healthy condition to +++ or ++++ in the partially spiked one) with the onset of the disease, but decreased to a much smaller amount (+) when the plant was fully spiked. On the other hand, oxalic acid, was comparatively high (+++) in the case of healthy specimens and relatively small (+ or traces) in the case of partially diseased or fully spiked plants.

Direct Examination of Acids in the tissues.—In addition to the methods already described, the one developed by Klein and Werner (*Z. physiol. Chem.*, 1925, **143**, 141) was adapted for this study. The technique as applied to sandal leaves may be described as follows :—

The material under examination (0.5 cm. sq.) is placed in a circular copper piece (E) (Fig. 1) 1 cm. diameter with a special loop and dried at 60° for 10 minutes after acidification with a micro-drop of phosphoric acid. The copper piece containing the dried mixture of acids is placed in position over iron filings in the apparatus (B) fitted with a condenser (C) to the flat bottom of which is fixed a round cover slip (D). The apparatus is evacuated (2 mm. pressure) and the contents heated slowly in an oil-bath.

On attainment of the stage at which an acid is known to sublime, (this was determined beforehand for each acid) the temperature was maintained constant for 10 minutes. The pressure was then released and the cover slip replaced by a fresh one. The evacuation was resumed and the temperature raised to a further stage and again maintained at that point for 10 minutes. The cover glass with the sublimate was again removed and a fresh one placed in its stead. In this manner, the fractional sublimation was continued, and the sublimates collected on the glasses were identified as their salts. Oxalic acid was identified as the calcium or strontium salt, succinic acid as the lead salt, malic and citric acids as silver salts and tartaric acid as its anhydride. By adopting this method it was possible to establish definitely the occurrence of succinic acid which is prominent in the spiked tissue

Fig. I
Micro Sublimation apparatus



A.—Oil bath for heating the apparatus.
B.—The outer vessel carrying in a groundin condenser.
C.—The groundin condenser.
D.—The cover slip on which the sublimed acid settles on cooling.
E.—The copper plate containing the precipitate under examination kept on iron filings.
T.—Thermometer.

but conspicuously absent from the healthy one. This observation being rather striking, specimens from four different localities were examined and the above observation confirmed (Table I).

TABLE I.

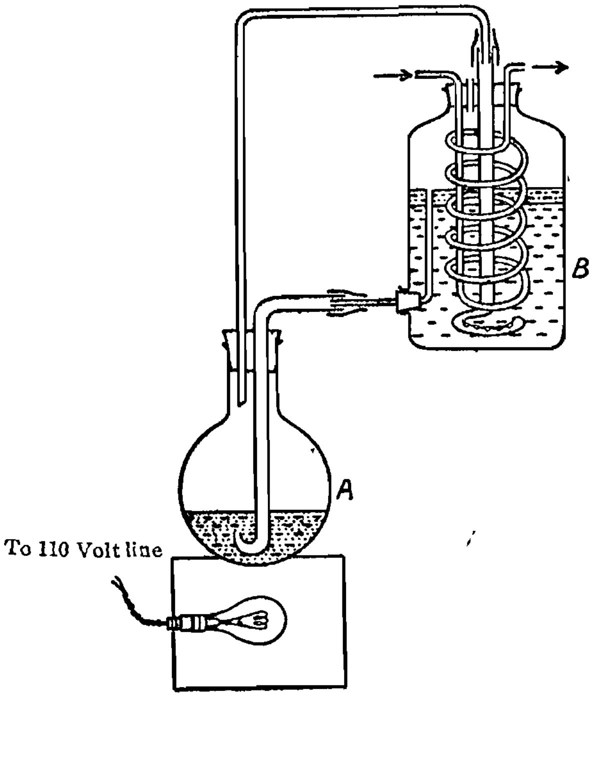
Succinic acid in leaf tissues.

Condition of tissue	Institute area	Ragihalli	Nognoor	Jawalgiri
Healthy	Absent	Absent	Trace	Absent
Spiked	+ +	+ +	+ +	+ +

Quantitative study of the distribution of organic acids.—Representative healthy and spiked trees were selected in three different localities. The leaf specimens used for each analysis were collected from single trees, the quantities examined varying between 1 and 3 Kgs. The specimens were treated according to the method of Vickery and Pucher (*J. Biol. Chem.*, 1931, **90**, 637). After removal of proteins, pectins and other interfering substances, the organic acids were precipitated as barium salts in preference to the lead ones and the free acids released by treatment with the minimum amount of sulphuric acid, 50 per cent. It was noticed that the final extracts thus obtained were usually more dark coloured in the case of diseased plants than in those of healthy.

The solution containing the free acids was extracted with ether for 320 hrs. in an apparatus specially devised for that purpose (Fig. 2). It consists of a receiver flask (A) of 1 litre capacity which contains

Fig. II
Extraction apparatus for liquids



the solvent and receives the extracted material. The vapour of the solvent passes through a tube ending in a spiral with a number of holes. It is absorbed in the container (B) a bottle of capacity 4 litres with a tube at the bottom which contains the acidulated solution to be extracted. The condensed solvent rises up in tiny bubbles extracting in its course the acids present. There is a syphon tube connecting the container to the bottom of the receiver so that as the level passes a particular point, the solvent flows down to the receiver. The condensation of the solvent was facilitated by cooling the container with ice (not shown in the figure). The condensed water was also drawn through ice so that the cooling was rendered still more efficacious. In this manner, the extraction proceeded continuously for several days without much loss of solvent. After the completion of the extraction, the combined ethereal extracts from the receiver and the container was distilled to remove the solvent and the residue esterified with acidified (2.5 per cent. HCl) absolute alcohol. After removal of the alcohol, the residue is taken up with ether, washed with water and neutralised with sodium carbonate. The sludge which then separates is again extracted with ether and the combined ethereal extracts washed, dried and the ether distilled. The residue was then subjected to fractional distillation and the quantities of organic acids represented by the different fractions determined. The results have been presented in Table II.

The various ethyl esters were further identified according to Franzen and Helwert (*Z. physiol. Chem.*, 1922, **122**, 46), as the corresponding hydrazides and the benzylidine derivatives. In all the cases the purity of the preparation was checked by the determination of mixed melting points with these derivatives of the pure acids. The respective nitrogen contents were also checked by micro as well as macro methods. The results have shown conclusively that malic and oxalic acids are present in very much larger quantities in the healthy sandal leaf than in the spiked one. On the other hand, succinic acid is prominent in the spiked tissue and either completely absent or present only in traces in the healthy one.

The residue left after extraction with water was further treated with sulphuric acid so as to liberate the organic acids from their insoluble salts. The mixture of free acids thus obtained was extracted with ether and the acids estimated in the manner already described. The results (Table II) again show that malic and oxalic acids were present as their corresponding salts to a much greater extent in the healthy sandal than in the spiked one.

TABLE II.

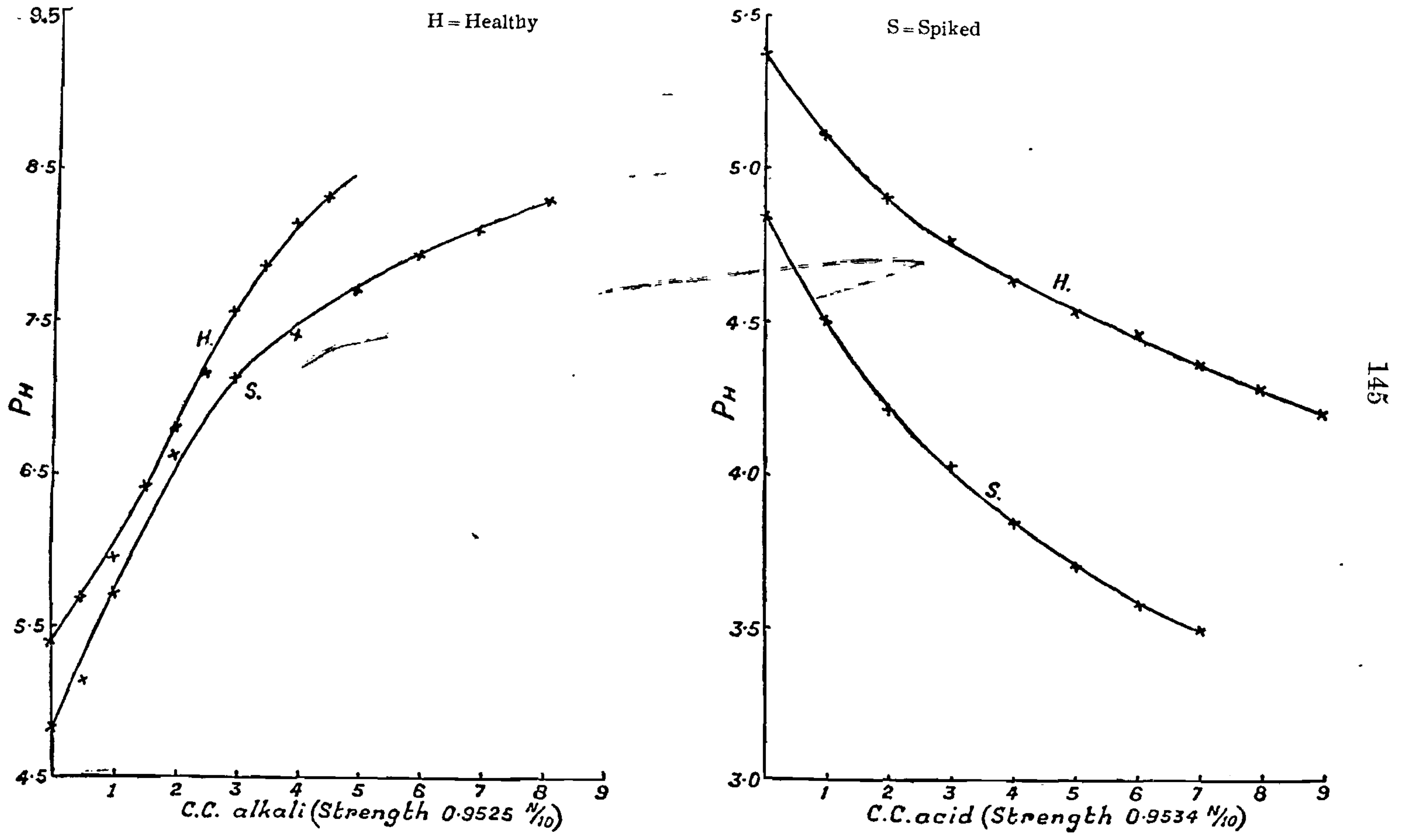
Distribution of organic acids in healthy and spiked sandal.

	Sources of Leaf Material											
	Ragihalli				Nognoor				Denkanikota Reserve			
	Healthy		Spiked		Healthy		Spiked		Healthy		Spiked	
	Wt. in gm.	Percentage on dry wt.	Wt. in gm.	Percentage on dry wt.	Wt. in gm.	Percentage on dry wt.	Wt. in gm.	Percentage on dry wt.	Wt. in gm.	Percentage on dry wt.	Wt. in gm.	Percentage on dry wt.
Fresh material taken ...	3800		1355		3400		1800		3500		1750	
Residue after water extraction ...	538		245		485		293		483		281	
Acids in the water soluble portion.												
Malic acid ...	2·05	0·19	0·44	0·10	2·45	0·21	1·10	0·17	2·45	0·23	0·82	0·12
Oxalic acid ...	0·38	0·04	Nil	Nil	3·38	0·29	0·13	0·02	2·24	0·21	0·30	0·04
Succinic acid .	Nil	Nil	2·40	0·53	0·06	0·005	4·0	0·62	Nil	Nil	5·06	0·75
Acids in the water extracted residue.												
Malic acid ...	0·51	0·05	0·21	0·04	1·17	0·10	0·45	0·07	1·49	0·14	0·48	0·07
Oxalic acid ...	5·01	0·48	1·52	0·30	6·41	0·55	1·43	0·22	6·19	0·58	2·04	0·30

Buffering capacities of tissue fluids from healthy and spiked sandal.—Since the hydrogen-ion concentration of the tissue fluid increases with the progress of the disease, some experiments were carried out with a view to determining the relative buffering capacities of the tissue fluids of sandal at various stages during the progress of the disease. Titrations were carried out against both acid and alkali and the quantities required to change the reaction of the different hydrogen-ion concentrations determined in each case. It was found (Fig. III) that though the fluids from the healthy and the spiked specimens behaved more or less alike when titrated against alkali or acid, those from the diseased showed weaker buffering capacity on the addition of increasing amounts of acid.

Fig. III

ALKALI & ACID TITRATIONS OF 5C.C. LEAF SAPS OF HEALTHY TREES AND TREES WELL ADVANCED IN SPIKE



With a view to verifying the above observation, the tissue fluids of specimens from four different localities were examined with regard to their response to the addition of acid or alkali and the quantities required for unit change of P_H on the acid or alkali side determined. The results (Table III) confirm the above observations.

TABLE III.

Titration values for healthy and diseased leaf saps (5 c.c.) against acids and alkalies.

Source of material	Initial P_H		Sulphuric acid in c.c. (N/10) required for a change of 1·25 P_H		Alkali required in c.c. (N/10) to change the reaction to P_H 8·3	
	Healthy	Spiked	Healthy	Spiked	Healthy	Spiked
Ragihalli ...	5·64	4·68	9·4	5·3	4·5	8·5
" ...	5·49	4·72	9·8	5·5	5·6	7·5
Uttarahalli ...	5·61	5·21	10·5	6·3	5·3	4·7
Nognoor ...	5·72	4·94	8·9	6·4	3·5	5·7
" ...	5·60	4·98	9·7	6·9	5·2	7·4
Denkanikota Reserve ...	5·63	5·05	10·1	6·5	4·3	6·6

Phosphates in health and disease.—Since these form important buffers in plant saps, the quantities present in the tissue fluids were determined according to Embden (*Z. physiol. Chem.*, 1921, **113**, 138). The results (Table IV) obtained in the case of five independent sets of healthy and spiked specimens show that the phosphate is invariably present to a greater extent in the fluid from diseased material than in that from the healthy.

TABLE IV.

Phosphoric acid contents of healthy and diseased leaf tissue fluids.

Condition of the material	P_2O_5 (as mg.) in 10 c.c. of tissue fluid				
	Specimens				
	I	II	III	IV	V
Healthy	7·48	13·48	5·12	8·48	10·42
Spiked	9·50	17·89	8·24	10·54	14·21

Organic acids and respiration.—In view of the important part played by organic acids as intermediary products in the respiration of plants, some preliminary trials were carried out estimating the evolution of carbon dioxide from healthy and spiked leaves according to the method of Dunlap (*Amer. J. Bot.*, 1930, **17**, 348) with slight modifications. The results have been presented in the following table (Table V).—

TABLE V

Material used	Duration of Experiment in hours	CO_2 production in mg. per gram of leaf			
		Mature leaves		Young leaves	
		Healthy	Spiked	Healthy	Spiked
Cut shoots with leaves ...	13	3·7	2·2	...	...
Do. ...	15	5·4	4·7	7·9	11·6
Do. ...	22	6·6	4·1	...	...
Leaves alone ...	17	6·1	8·1	10·9	16·2

The results represent the averages of a number of determinations.

DISCUSSION.

It is well known that organic acids are formed as intermediary products of the respiratory metabolism of the plant cell. These processes serve to release the energy necessary for the growth of the plant. The substrates generally concerned in the transformations are the carbohydrates, the fats and the proteins which lead to the formation of the several acids found in plants.

The study of organic acids in plant parts has formed the subject of a number of important inquiries. The organic acid contents of fruits have been investigated by Franzen and his co-workers (*Z. physiol. Chem.*, 1921, **115**; *ibid.*, 1922, **121**; *ibid.*, 1923, **127**) and by Nelson and his co-workers (*J. Amer. Chem. Soc.*, 1925, **47**; *ibid.*, 1927, **49**). In a recent communication, Vickery and Pucher (*loc. cit.*) describe their studies on the distribution of organic acids in tobacco leaves. The relation of acidity to plant growth was investigated by Hurd (*J. Agric. Res.*, 1923, **25**, 457) who observed a parallelism between acidity and vegetative vigour in corn. In a later publication he reported an

increase in acidity in the final stages of growth in the wheat plant (*ibid.*, 1924, **27**, 725).

A remarkable feature of the spike-disease of sandal is that with the onset of the disease the reaction became more alkaline than that of the one in the healthy condition. This continues only for a period after which the hydrogen-ion concentration steadily increases becoming distinctly more acid in the advanced stages of spike. Another striking transformation resulting from the diseased condition is the marked fall in malic and oxalic acid contents together with a distinct increase in succinic acid which is practically absent from the tissues and the tissue fluids of the healthy plant (Table II). Oxalic acid is present mostly in the insoluble form as the calcium salt and since the spiked plant is always deficient in calcium as shown by the author (*loc. cit.*), the deficiency in this acid may be traceable to the diminished quantity of calcium.

The foregoing studies have also brought out the importance of the micro-sublimation technique. It has been of much assistance in the identification and estimation of the different organic acids. It has helped to show conclusively the presence of succinic acid in the spiked plant (Table I). With the extension of this technique, it should be possible to devise a series of quantitative methods suitable for the identification and the handling of substances of biological products which are present only in the minutest quantities.

The abnormal change in reaction characteristic of the onset of the disease is supported by similar observations by other workers. Wagner observed a similar condition in the case of plants infected with a variety of pathological organisms (*Zentr. f. Bakt. Abt.* II, 1916, **44**, 708). He observed an increase in P_H values immediately after the infection. The reaction tended to become acid with the progress of the disease. In case of recovery, the reaction of the infected plants became normal, but with the persistence of infection, there was a temporary rise in the P_H value which dropped suddenly at the stage prior to the death of the plant. Hurd (*loc. cit.*) observed an abnormal increase in the hydrogen-ion concentration in wheat plant attacked by mildew. The variation in resistance could not, however, be correlated with either the initial hydrogen-ion concentration or the titratable acidity. Furthermore, the acid reaction does not appear to hinder the attack of stem rust; nor does a low acidity predispose the plants to disease. Again Hurd and Karrer (*Amer. J. Bot.*, 1925, **12**, 259) could not trace any correlation in hydrogen-ion concentration or titratable acidity and resistance of wheat to infection by *Tilleti Tritici*: in a like manner the differences in hydrogen-ion concentration were not, in any way,

correlated with immunity to wart disease in potato (Weiss and Harvey, *J. Agric. Res.*, 1921, **21**, 590). In the case of spike, on the other hand, a distinct formation of succinic acid and the diminution in the hydroxy acids (Table II) might be in some way related to the increased hydrogen-ion concentration particularly in the advanced stages of the disease, while the presence of the corresponding mono and dihydroxy acids as also the neutralized oxalic acid in the tissue and the tissue fluids of healthy sandal and in the diseased specimens from partially spiked plants, may be considered to have imparted a less acid reaction.

The titration values for the tissue fluids of healthy and spiked sandal (Table II) bring out certain important features of the disease. The alkali required to neutralize the tissue fluid in the partially spiked plant is less than that of the healthy. With the progress of the disease the amount of alkali required increases in quantity in the advanced stages of the disease. On the other hand the acid required to bring about unit change shift of P_H in the diseased condition is less than in the normal. It can be seen from the titration curves presented in Fig. III, that the character of the curve is steeper in the affected specimens. In the case of acid titration this indicates a greater P_H change on the addition of small quantities. It appears from this that the spiked leaf sap is less buffered than the healthy one. The significantly low initial acidity may explain the increased alkali requirement in such cases. In this connection it may be mentioned that sulphuric acid was specially chosen for the titrations. The application of poorly ionised acids like acetic or oxalic acid would not only require large quantities of these acids for bringing about even small changes in the hydrogen-ion concentration, but would also introduce error due to high dilution. On the other hand, the employment of a highly ionised acid such as hydrochloric would bring about sharp changes through the addition of even small quantities, thus rendering it difficult to notice subtle differences between the different specimens.

The phosphate content of the tissue fluids does not provide sufficient information regarding the nature of the factors leading to increased acidity of the fluid from the spiked material. The presence of consistently larger quantities of phosphates in the spiked material than in the healthy, is rather significant (Table IV). It is probable that phosphorus exists as the acid salts of alkalies or alkaline earths or even as free phosphoric acid and this may explain to some extent the increased alkali requirement of the diseased leaf sap. Some preliminary experiments on the estimation of phosphorus present in organic form were carried out by extracting leaf powders with different solvents. The results showed that more phosphorus was present in that

form in the healthy tissue than in the spiked one. The relation of this observation to buffering capacity is, however, still obscure and further work is in progress to establish the rôle of phosphates in conditions of health and disease.

The respiration studies show that the spiked leaf gives off per unit weight more CO_2 than the healthy. On the other hand the shoots bearing the leaves show a distinctly reduced respiratory activity in the case of spiked specimens than that of the healthy (Table V). This would indicate that portions of the shoot other than the leaves do not show any appreciable respiratory activity in the case of diseased specimens.

Observations on the respiratory activity of sandal in health and disease are supported by a number of previous investigators. Weimer and Harter (*J. Agric. Res.*, 1921, **21**, 627) observed that in the case of sweet potatoes attacked by *Rhizopus tritici*, the diseased portion gave off 6.3-7.8 times as much CO_2 per unit weight as the healthy one. Davis (*Bot. Gaz.*, 1926, **81**, 323) noticed a striking increase in CO_2 production in the case of potato tubers with the onset of Blackheart. Dunlap (*loc. cit.*) observed a decrease in the CO_2 production in plants suffering from yellows and mosaics, but in the case of young tobacco leaves affected by mosaics his conclusions correspond to those in the case of young sandal leaves, in that the respiratory activity is high.

It would appear that in many of the previous studies particularly with those on fungus disease the apparent increased respiratory activity would be accounted for by the respiration of the parasite itself. Since the nature of the parasite (or the infective principle) in the case of spike is still obscure it is not possible to state at this stage as to what extent the previous observations can be applied to the present study. The relation of the nature and the distribution of organic acids to the respiratory activity is still comparatively obscure. The predominance of succinic acid in the case of spiked leaf as compared with those of oxalic and malic acids in the healthy one would suggest that some internal reduction takes place in the diseased condition. The increased CO_2 production may, however, be related to the larger quantities of soluble carbohydrates in the spiked leaf, as has been previously shown by the author (*J. Indian Inst. Sci.*, 1928, **11A**, 93). The above explanation would however require further experimental support.

It is generally believed that organic acids arise through incomplete oxidation of hexoses in the respiratory processes of the cell. The investigations of Ruhland and Wetzel (*Planta*, 1926, **1**, 558) and other workers would suggest a close correlation between the formation of organic acids and transformations of plant proteins. Kostychew

(*Plant Respiration*, edited by Lyons, 1927) and Onslow (*Principles of Biochemistry*, part I, 1931) have discussed the possibilities of several methods of formation of organic acids. Kostychew holds the view that acids arise as either transformation products of amino-acids or as intermediate products in the course of transformation of sugars to amino-acids in the synthesis of proteins. The researches of a number of previous workers (e.g., Neubauer and Fromherz, *Z. f. physiol. Chem.*, 1911, **70**, 326; Neuberg and Ringer, *Biochem. Zeit.*, 1918, **91**, 131) have shown that succinic acid is formed by the yeast from glutamic acid by a process of oxidative deamination. In a similar manner the presence of oxalic and malic acids would be traceable to aspartic acid. The extent to which the increased malic and oxalic acids in the healthy condition and the exclusive presence of succinic acid in the spiked tissues are traceable to the above two amino acids is still obscure. Further researches are in progress to throw light on this aspect of the problem.

One of the main objects of the present study was to trace the resistance offered by sandal occurring in certain localities or in association with certain hosts to the hydrogen-ion concentration and the buffering principles present in the tissue fluids. The factors which determine the resistance offered by plants to disease has been the subject of a number of important inquiries by Nobecourt (*Contribution á l'étude de l'immunité chez les Végétaux*, 1928) and Carbone and Arnaudi (*L'Immunita Nelle Piante*, 1930). According to these authors the defensive mechanism of plants against parasitic attack is traceable to deficient chemical entities such as hydrogen-ion concentration, sugars, organic acids and tannins etc. Comes (*Reale Istituto d'Incoraggiaments di Napole*, 1916) first showed that acidity was a very important factor determining immunity of plants. Mumford studied the relation of hydrogen-ion concentration of tissue fluids of sugar beet in relation to 'curly top' disease, but could not find any significant difference between those from healthy and diseased materials or from resistant and susceptible varieties (*Ann. Appl. Biol.*, 1930, **17**, 35). The more recent observations of Walker and his co-workers (*J. Biol. Chem.*, 1929, **81**, 369; *ibid.*, 1929, **84**, 719) have shown however that the resistance in certain varieties of onions to the smut disease is traceable to the presence of protocatechuic acid which is present in the scales of red and yellow varieties which are known to be resistant but absent from the white ones which are susceptible to the disease. The present study gives no clear indication of the relation of organic acids to the resistance offered by sandal to spike-disease. The extent to which the presence of tartaric, malic and oxalic acid, contribute towards resistance to spike and that of succinic acid to one of susceptibility is still obscure. These and related problems would form subjects of a later communication.

SUMMARY.

1. Leaves from healthy and diseased sandal from different localities were examined by micro and macro chemical methods for the several organic acids present in them. It was observed that the tissues and the tissue fluids from the former contain more malic and much larger quantities of oxalic acid than the latter. On the other hand, succinic acid was prominent in the spiked tissues and tissue fluids while being completely absent from or present only in traces in the healthy material.

2. An apparatus for extracting large quantities of solutions with light solvents has been described.

3. Titrations against acid or alkali showed that the disease brings about a disturbance in the buffering capacity of the tissue fluid.

4. The tissue fluids from spiked sandal contain more phosphates than those of the healthy.

5. Carbon dioxide production is more intense in the case of spiked leaf tissue than that of the healthy. On the other hand cut shoots (with leaves) from the spiked plant gives out less carbon dioxide per unit weight than the corresponding healthy specimens.

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THE SOLUBILITY OF SILVER CHLORIDE

By P. C. Dave and K. R. Krishnaswami.

The solubility of silver chloride in water, which at 25° is of the order of 2 milligrams per litre, is sufficiently great to cause appreciable errors in accurate analytical work if not allowed for, while in atomic weight determinations involving the precipitation of silver chloride, a knowledge of the solubility is essential. The relation between the solubility determined by the conductivity method and by other means is also of theoretical importance and it is therefore not surprising to find that numerous papers on the subject have been published. Of these the majority refer to the solubility in water, and very little information is available regarding the solubility in nitric acid or salt solutions in which silver chloride is usually precipitated.

The available data for water are shown in Table I, the solubility being expressed in moles per litre x 10^5. For the purpose of comparison, the values at 25° are given in column 6; these when not recorded directly, have been obtained either by interpolation from the results at other temperatures or, when these are not available, by assuming that the change in solubility in the region under consideration is the same as the one found in our experiments.

The variations in these figures are distinctly larger than might be expected and suggest that factors are present which have not been taken into consideration when presenting the results. One possibility is that some of the methods are inherently wrong or are subject to constant errors. A comparison of results obtained by the same method shows however that this is not a sufficient reason and it is much more probable that the explanation is to be found in the varying solubility of silver chloride according to its method of preparation and subsequent treatment. As long ago as 1871, Stas (*Compt. rend.*, 1871, **73**, 1000) observed that precipitated silver chloride could exist in four different forms which he designated as gelatinous, flocculent, powdery or granular. The solubility of these varied from 19mg./l. for the gelatinous variety to 0.02 mg./l. for the granular. Although the results obtained by Stas cannot be considered very accurate and his observation regarding the low solubility of the granular variety has not been confirmed, his remarks suggest that unless similar specimens are employed for solubility determinations, the values obtained will not be comparable.

Richards and Wells (*J. Amer. Chem. Soc.*, 1905, **27**, 485) observed that if silver chloride was filtered immediately after precipi-

TABLE I.

Solubility of silver chloride in water

No.	Authors	Method employed	Temperature	Solubility	Solubility at 25°	Reference
1	Holleman ...	Conductivity ...	13·8 26·5	0·97 1·82	1·74	*Z. physikal Chem.*, 1893 **12,** 132.
2	Kohlrausch and Rose.	do. ...	2·0 10·0 18·0 26·0 34·0 42·0	0·53 0·68 1·06 1·56 2·11 2·82	1·50	*ibid.*, 1893, **12,** 242.
3	Böttger ...	do. ...	19·95	1·07	1·34	*ibid.*, 1903, **46,** 603.
4	Kohlrausch ...	do. ...	18·0	1·12	1·56	*ibid.*, 1905, **50,** 356.
5	Do. ...	do. ...	1·55 4·68 9·97 17·51 18·0 25·86 34·12	0·39 0·46 0·62 0·91 0·93 1·35 1·91	1·30	*ibid.*, 1908, **64,** 168.
6	Melcher ...	do. ...	18·0	1·05	1·42	*J. Amer. Chem. Soc.*, 1910, **32,** 50.
7a	Van Rossem ...	do. ...	18·0 25·0	1·04 1·43	1·43	*Chem. Weekblad.*, 1912, **9,** 396.
7b	Do. ...	E. M. F. ...	18·0 25·0	1·02 1·43	1·43	*idem.*
8	Goodwin ...	do. ...	25·0	1·25	1·25	*Z. physikal Chem.*, 1894, **13,** 645.
9	Thiel ...	do. ...	25·0	1·41	1·41	*Z. anorg. Chem.*, 1900, **24,** 57.
10	Abegg and Cox...	do. ...	25·0	1·25	1·25	*Z. physikal Chem.*, 1903, **46,** 1.
11	Pinkus and Berkolaiko.	E. M. F. and Nephelometric.	25·0	1·42	1·42	*J. Chim. physique*, 1930, **27,** 364.
12	Scott and Johnson.	Nephelometric ...	0·5	0·50	...	*J. Amer. Chem. Soc.*, 1930, **52,** 3586.
13	Rolla ...	Optical ...	18·0	1·20	1·57	*Atti. R. Acad. Lincei.* 1913, **22,** 2.
14	Hill ...	Volumetric ...	25·0	1·60	1·60	*J. Amer. Chem. Soc.* 1908, **30,** 74.
15	Glowczynski ...	do. ...	25·0	1·20	1·20	*Kolloidchem. Beih.*, 1914 **6,** 147.
16	Whitby ...	Colorimetric ...	21·0	1·07	1·29	*Z. anorg. Chem.*, 1910 **67,** 108.

No.	Authors	Method employed	Tempe-rature	Solubi-lity	Solubi-lity at 25°	Reference
17	Popoff and Neuman.	Tyndal beam ...	25·0	1·278	1·278	*J. Physical Chem.*, 1930, 1853.
18	Eversole and McLachlan.	do. ...	25·0	1·52	1·52	*J. Amer. Chem. Soc.*, 1932, **54**, 867.
19	Neuman ...	do. ...	25·0	1·273	1·273	*ibid.*, 1932, **54**, 2195.

tation the filtrate contained 1.5 mg./1. of silver chloride, whereas if filtration was deferred for several days, the quantity in solution fell to 1.1 mg./l. The difference is almost certainly due to aggregation of the particles and this aspect of the question has been discussed by Glowczynski (*loc. cit.*) who pointed out that a decrease in the degree of dispersion would lower the solubility.

The first seven determinations given in Table I were all made by the conductivity method, but the first two of them were not carried out under favourable experimental conditions. Determinations Nos. 3 and 5 were conducted with silver chloride precipitated from cold dilute solutions and washed with cold water. The conductivity was determined at closely regulated temperatures and the solution was protected from actinic light during the experiments. Böttger's value was the mean of thirty-two separate and concordant determinations and is in good agreement with the value 1.04 interpolated from Kohlrausch's figures. Melcher and Van Rossem's determinations (Nos. 6 and 7) appear to have been made with as much care as those of Kohlrausch or Böttger, but the silver chloride employed was obtained by precipitation from boiling solutions, washing with hot water and drying. The results of Melcher are in agreement with those of Van Rossem but not with those of Böttger or Kohlrausch.

Determinations Nos. 7b, 8, 9 and 10 were carried out by the electromotive force method, but except in the case of No. 7b no details are given of the way in which the silver chloride was prepared. Abegg and Cox made a large number of determinations and obtained the value 1.25×10^{-5} *M./L.* (moles. per litre) identical with Goodwin's figure; Van Rossem, however, after a prolonged series of measurements arrived at the figure 1.43×10^{-5} *M./L.* identical with the value he had obtained by the conductivity method and in good agreement with the result obtained by Thiel. It appears highly probable that the discrepancy between the two sets of values is due to differences in the methods of preparing the silver chloride. The wide difference between the results of Glowczynski and of Hill who both made use of the thiocyanate method is probably to be ascribed to the same cause.

Scott and Johnson and also Pinkus and Berkolaiko employed the nephelometric method. The former authors described their silver chloride as 'flocculent' but as they worked only at 0.5° it is not possible to compare the results. Pinkus and Berkolaiko prepared silver chloride by the addition of a slight excess of 0.1N hydrochloric acid to silver nitrate solution and boiling the mixture. The precipitate was washed with cold water and the solubility determined within five hours of precipitation. Their results by the nephelometric and E.M.F. methods were in good agreement.

Popoff and Neuman and subsequently Neuman made several determinations by observing the concentration at which the Tyndal beam due to turbidity just made its appearance. A very high order of accuracy, viz. $1 \times 10^{-8} M./L.$ was claimed for this method, and the two sets of results are in close agreement, but the mean value 1.273×10^{-5} is at great variance with the figure 1.52×10^{-5} obtained by Eversole and McLachlan under similar experimental conditions. It therefore appears probable that the method involves some source of error which has not yet been determined.

The available data for the solubility of silver chloride in nitric acid and sodium nitrate of various concentrations are given in Table II. The concentration of nitric acid and sodium nitrate is expressed in moles. per litre and the solubility of silver chloride in $M./L \times 10^{-5}$

TABLE II.

Solubility of silver chloride in nitric acid and sodium nitrate solutions.

Glowczynski	25°	HNO_3	0·0005	0·001	0·01	0·3
		AgCl	1·15	1·19	1·24	1·54
Eversole and McLachlan.	25°	HNO_3	0·0238	0·119	0·238	0·52
		AgCl	1·54	1·62	1·70	2·35
Scott and Johnson ...	0·5°	HNO_3	0·11	0·24	0·49	0·99
		AgCl	0·628	0·698	0·711	0·732
Mulder	15–20°	$NaNO_3$	0·047	0·093	0·355	...
		AgCl	6·7	9·3	17·6	...

The results of Mulder (*Die Silberprobirmethoden, Leipzig,* 1859) are very old and the exact temperature is not specified. They serve to indicate, however, that the solubility of silver chloride is largely increased by the addition of sodium nitrate, and the values are of the same order as those we have obtained.

The solubility of silver chloride in aqueous solutions of nitrates has also been studied by Popoff and Neuman (*loc. cit.*) but their work, was confined to solutions of very low concentrations of the order

0.00001-0.01 M. as the object in view was the study of the applicability of modern theories of strong electrolytes to such solutions.

To supplement these results we have determined the solubility of silver chloride in water and in solutions of nitric acid and sodium, potassium and ammonium nitrates over a range of temperature from 0 to 50°.

EXPERIMENTAL.

Preparation and purification of materials.—The pure water used in all the experiments was obtained by redistillation of the laboratory distilled water from a dilute, slightly alkaline solution of potassium permanganate.[1] The distillation was carried out in an all-glass apparatus, with a ground-in condenser of fused transparent silica. The water was prepared immediately before use and stored in bottles of resistance glass. It was found to be halogen free when tested in a nephelometer and had a specific conductivity of about 1×10^{-6} mhos.

The silver employed for the preparation of silver chloride was of a high degree of purity (99.996 per cent.) and when dissolved in pure nitric acid the solution was always clear and halogen-free.

The alkali nitrates were purified by repeated evaporation to dryness with concentrated nitric acid to remove traces of chloride which were found to be present in some of them. Three or four digestions with nitric acid always yielded halogen-free material which was then recrystallised and employed for preparing solutions of the required concentrations.

The silver chloride required for the solubility determinations was prepared as follows:—An accurately weighed quantity of silver (1.0788 g.) was placed in a conical flask which was provided with a funnel to collect the spray. Dilute nitric acid (20 c.c. of 1 : 3) was introduced into the flask which was then gently warmed. After the silver had dissolved, the liquid in the flask was diluted by the addition of 100 c.c. of water through the funnel, and boiled for about 10 minutes. The liquid was then cooled, transferred to a litre measuring flask and made up to the correct volume. A solution of sodium chloride of exactly equivalent concentration to the silver solution, i.e. containing 0.5846 g. of NaCl per litre was next prepared. Equal volumes (200 c.c.) of the solutions of silver and sodium chloride were mixed in a stoppered conical flask and shaken vigorously for about 10 minutes,

Shaking was continued for 5 minutes at intervals of ½ hour for a period of about 10 hours and the flask then set aside. At the end of about 24 hours it was found that the silver chloride had settled completely leaving the supernatant liquid quite clear and it was possible to pour off all but 20 c.c. 500 c.c. of water was then poured into the flask, shaken with the precipitate periodically, allowed to settle and poured off. The silver chloride remaining after four such washings was considered to be sufficiently pure. When the solubility was to be measured in nitric acid or a salt solution, a portion of the solution was used for the last two washings instead of water. The precipitation as well as the washing of the silver chloride was carried out at room temperature in a room illuminated by ruby light and the precipitated silver chloride always remained moist.

In order to determine the solubility, the moist silver chloride (0.1-0.2 g.) and the solvent (100 c.c.) were kept in separate vessels in a thermostat at the required temperature for about ½ hour, after which the silver chloride was added to the solvent and agitated frequently with a glass stirrer during 4 hours. After standing for periods which varied from 24 to 48 hours a portion of the clear supernatant liquid was decanted into another vessel kept in the thermostat and used for determining the solubility. It was found that the solubility did not vary with the period of standing. For temperatures from 25 to 30° the thermostat employed was capable of being regulated to within 0.02°, but for higher temperatures the variation reached 0.5°. For temperatures below that of the room, a large Dewar vessel containing cold water was employed and the temperature regulated by slowly adding iced water. In this case also the variation was 0.5°.

Since the solubility of silver chloride in the solvents employed increases appreciably with temperature it was necessary to dilute all solutions prepared at temperatures higher than that of the room to prevent separation of silver chloride when they were cooled for examination. This was done as follows :—The saturated solution of silver chloride was added to a known volume of water contained in a calibrated 125 c.c. measuring flask with 0.1 c.c. graduations on the neck until the level was near the top of the graduations, and the flask was stoppered, shaken and allowed to attain the temperature of the laboratory. The level of the liquid was then read, giving the quantity of saturated solution added, volume changes on mixing being negligible even with the most concentrated solutions employed. The quantity of water in the flask was sufficient to prevent precipitation on cooling and was calculated from the results of preliminary solubility determinations.

Estimation of the dissolved silver chloride. The nephelometer employed for the determination of silver chloride was of the Kober type with a Klett top-reader. Plain glass cups 10 cm. long provided with opaque glass bottoms contained the solutions during measurements of opalescence.

The standard solutions of silver chloride required for comparisons of opalescence were prepared thus. 2.00 c.c. of the standard silver nitrate solution was accurately measured by means of a micro-pipette, mixed with the same volume of the standard solution of sodium chloride and shaken vigorously. The precipitated silver chloride was just redissolved by gradual addition of a slight excess of very dilute ammonia and the ammonical solution so obtained was diluted to exactly 250 c.c. Each c.c. of this solution contained 0.00318 mg. of silver chloride. In a few cases where higher concentrations of silver chloride had to be estimated, standard solutions with higher concentrations of silver chloride were prepared.

The generation of opalescence for examination in the nephelometer was performed as follows :—An accurately measured quantity (2.00-5.00 c.c.) of the solution to be examined was introduced into the nephelometer cup, diluted with water (2-5 c.c.) acidified with 0.5N. nitric acid (1-2 c.c.) and the silver chloride precipitated by slow addition of 1N. sodium chloride (1-2 c.c.) from a pipette, the liquid in the cup being stirred by a thin glass rod bent into a ring at one end. This procedure is recommended by Scott and Moilliet. (*J. Amer. Chem. Soc.*, 1932, 205). An identical procedure was adopted for generating opalescence in the standard solution of silver chloride. The concentrations of the two solutions were made approximately equal so that the heights of the liquids in the two cups were of the same order when measured. All the precautions necessary for accurate work (Richards and Wells, *loc. cit.*) were strictly observed.

The results obtained in the various experiments carried out in different concentrations of the solvent and at various temperatures are assembled in Tables III and IV. The mixed solvents employed in the experiments detailed in Table IV were prepared by mixing the necessary amounts of the two solvents of known molarities. The table gives the molarity of each of the nitrates and the solubility S of the silver chloride.

Each of the values given in the tables is the mean of two or three independent determinations. In each of these the value was deduced from at least four nephelometer readings which were found to be concordant to about 1 part in 400. The variation between different determinations was about 0.5 per cent.

TABLE III.

Solubility of silver chloride, M./L. $\times 10^{-5}$.

Solvent	Molarity	0°	8°	18°	25°	30°	40°	50°
[1] Water ...	...	0·49	...	1·01	1·39	1·69	2·63	3·82
HNO_3 ...	0·11	0·60	...	...	1·51	...	...	...
	0·48	0·71	0·89	1·38	1·64	1·84	3·98	7·69
	0·81	0·77	...	...	1·68	...	...	...
	1·00	0·79	...	...	1·74	...	...	...
[2] $NaNO_3$ ...	0·0001	...	...	...	...	1·69	...	...
	0·001	...	...	...	...	1·91	...	...
	0·01	...	...	...	...	2·80	...	...
	0·05	...	...	...	7·35	...	...	...
	0·10	1·35	3·00	4·94	10·1	10·1	10·2	10·8
	0·50	...	...	...	13·5	...	...	...
	0·75	...	...	...	15·5	...	...	...
	1·00	...	...	...	17·1	...	...	...
	3·00	...	...	...	17·5	...	...	...
	Sat. soln.	...	...	...	18·4	...	...	...
KNO_3 ...	0·10	0·79	0·84	1·42	2·71	2·93	4·30	6·05
	0·50	...	...	...	...	5·15	...	...
	0·75	...	...	...	...	5·92	...	...
	1·00	...	...	...	...	6·93	...	...
NH_4NO_3 ...	0·10	0·68	0·82	1·44	3·27	3·28	4·84	6·75
	0·50	...	...	...	5·23	...	...	...
	0·75	...	...	...	6·33	...	...	...
	1·00	...	...	...	7·40	...	...	...

TABLE IV.

Solubility of silver chloride in mixed solvents at 30°.

KNO_3	+ HNO_3	S.	NH_4NO_3	+ HNO_3	S.	$NaNO_3$	+ HNO_3	S.
0·10	0·25	2·94	0·10	0·25	3·29	0·10	0·25	10·1
0·10	0·50	2·98	0·10	0·50	3·32	0·10	0·50	10·2
0·50	0·25	5·19	0·50	0·25	5·25	0·50	0·25	13·5
0·50	0·50	5·21	0·50	0·50	5·26	0·50	0·50	13·8

The solubility of silver chloride in water in the range studied increases with rise in temperature but it was found that the results could not be expressed by a parabolic equation of the usual type. The expression

$$\log S = 0{\cdot}01816\, t - 0{\cdot}318$$

calculated by the method of least squares, S being the solubility, fits the experimental results within the limit of error as shown in Table V.

[1] The solubility of silver chloride in water at 10° was found to be 0·72.

[2] The solubility of silver chloride in 0·1 M. solutions of sodium nitrate at 22 and 23·5° was found to be 5·86 and 6·56 respectively.

Fig. 1.

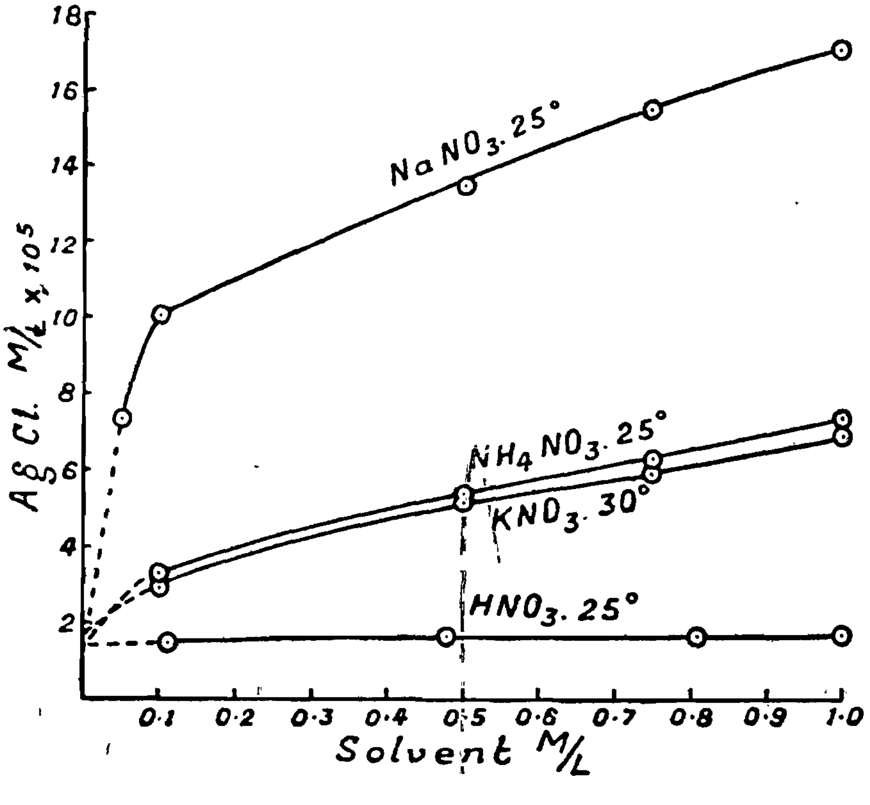

TABLE V.

Temp.	0	10	18	25	30	40	50
S calc.	0·48	0·73	1·02	1·37	1·69	2·56	3·89
S found.	0·49	0·72	1·01	1·39	1·68	2·63	3·82

The addition of nitric acid or nitrates in small quantities up to a concentration of 0.1 M. results in a marked increase in solubility as may be seen from fig. 1 which shows the results at 25°. On increasing the concentration, the solubility continues to rise but not nearly as rapidly as with more dilute solutions. The effect with nitric acid is the least and with sodium nitrate the greatest. Potassium and ammonium nitrates behave in a similar manner to each other.

The temperature-solubility curves are somewhat remarkable owing to their irregular nature as may be seen from fig. 2. It will be observed that in two cases an abrupt rise is found very near to 25°. As this is the lowest temperature at which an automatically controlled

from tables of activity coefficients (Lewis and Randall, *Thermodynamics*, p. 382) x may be calculated for the various solutions examined. The results so obtained are given in Table VI.

TABLE VI.

Complexity of silver chloride solutions.

Solvent	Conc.	Temp.	$S \times 10^5$.	γ_{Ag}.	$\alpha_{Ag} \times 10^6$	x
$NaNO_3$	0·1	0	1·35	0·77	2·27	0·78
	,,	18	4·94	,,	2·65	0·93
	0·05	25	7·35	0·80	3·27	0·94
	0·1	,,	10·07	0·77	2·48	0·97
	0·001	30	1·91	0·97	14·25	0·23
	0·01	,,	2·81	0·90	10·47	0·59
	0·1	,,	10·09	0·77	3·41	0·96
	0·1	40	10·23	,,	8·79	0·89
	,,	50	10·78	,,	17·60	0·79
NH_4NO_3	0·1	0	0·69	0·77	4·43	0·17
	,,	18	1·44	,,	9·11	0·18
	,,	25	3·27	,,	7·65	0·70
	,,	30	3·29	,,	10·44	0·59
	,,	40	4·84	,,	18·6	0·50
	,,	50	6·75	,,	28·12	0·46
KNO_3	0·1	0	0·79	0·77	3·88	0·36
	,,	18	1·42	,,	9·22	0·16
	,,	25	2·71	,,	9·21	0·56
	,,	30	2·93	,,	11·71	0·48
	,,	40	4·30	,,	20·91	0·37
	,,	50	6·18	,,	30·68	0·36

These figures indicate that (1) the degree of complexity is a maximum at 25°, (2) it is much greater for solutions of sodium nitrate than for ammonium and potassium nitrates and (3) in the case of sodium nitrate it increases with the concentration of the solution at constant temperature.

SUMMARY

1. The solubility of silver chloride in water and in aqueous solutions of nitric acid and the nitrates of sodium, potassium and ammonium has been determined by a nephelometric method over a temperature range 0-50° under conditions approximating to those prevailing during analytical work.

2. The solubility in water at 25° has been deduced from conductivity measurements giving the value 1.373×10^{-5} M/L as compared with $1.388 \times 10^{-5} M/L$ by the nephelometric method.

3. The solubility of silver chloride in solutions of sodium nitrate has been found to be much higher than in the other solutions studied and this is due to the existence as complex ions of a large proportion of the silver ions.

We desire to express our gratitude to Professor H. E. Watson for his constant interest and valuable suggestions during the progress of this work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL (*SANTALUM ALBUM*, LINN.)

PART I. Nature and extent of parasitism.

By Y. V. Sreenivasa Rao.

Sandal belongs to the Natural Order *Santalaceae,* which comprises an entire class of root parasites and of which about two hundred species are known to occur widely distributed all over the world, in the temperate as well as tropical forests. Of these, sandal is practically the only economic species, the aggregate annual revenue received from it amounting to several lakhs of rupees.

That sandal is a root parasite was first recognised by Scott in the year 1871, but the practical significance of this fundamental fact is still not adequately appreciated. Fuel exploitation is allowed in sandal-bearing areas which results in gradual depletion of the host plants of the parasitic sandal. Afforestation of sandal areas with suitable species of hosts is essential for successful sylviculture of sandal.

Rama Rao (Indian Forest Record 1910, **2**, Part V, 159) gives a list of some 140 species of plants which he found to have been haustorised by sandal. The fact that sandal has made its haustorial connection with a particular species is not, however, a criterion for the latter being a suitable host plant. The extensive pot culture trials carried out by Sreenivasaya at the nursery of the Indian Institute of Science have, in fact, established that there is great variation in the "host value" of various species of plants. Thus, whereas leguminous hosts generally nourish the sandal to rapid and luxurious growth, plants of the phyllanthus species make bad hosts. It is not possible to raise sandal with *Phyllanthus embellica* though a haustorial connection may, with some difficulty, be established between the host and the parasite.

An exhaustive study of the haustorium was conducted by Barber (*Indian Forester,* 1904, **30**, 545; *ibid.,* 1905, **31**, 189; *Mem. Dept. Agric. India, Bot. Ser.,* Vol. I, No. I, Parts 1 and 2) who also investigated the conditions for its formation by sandal. In view of the economic importance of the plant and the serious nature of the spike disease, a closer knowledge of the physiology of sandal is necessary.

The present communication deals with two aspects of the problem :—(1) the extent to which sandal is dependent on its host in regard to its mineral nutrients and (2) the physico-chemical relationship existing between the host and the parasite.

EXPERIMENTAL.

Material.—Sandal seedlings numbering one hundred, were transplanted into pots made up with equal quantities of manure and soil. Into one set of pots, *Acacia farnesiana* was introduced as the host, while an equal number (50) were left as controls without any host plant.

It was observed that the seedlings in association with the host soon developed a healthy, green flush and put on rapid growth as contrasted with controls which made no appreciable progress and the leaves of which turned to an unhealthy pale yellow (*vide* photograph).

Six months after transplanting, when the difference between the plants with and without hosts became quite manifest, they were carefully removed from the pots, washed free from mud and adhering dirt and divided into three portions, leaves, stems and roots. The specimens were dried over calcium chloride in a vacuum desiccator. They were then powdered to pass a 40-mesh sieve and preserved in bottles for analysis.

Methods.—Total nitrogen was estimated according to the method of Kjeldahl as modified by Gunning to include nitrates. Total ash, phosphoric acid, potash, calcium, iron and alumina were estimated according to the standard methods of A.O.A.C. (1925). The results have been presented in Table I in which two independent sets of observations showing the possible range of values have been given.

It may be seen from the above that the different parts of sandal nourished by hosts were consistently richer in nitrogen, phosphorus and potash than the corresponding portions of plants without hosts. On the other hand, the latter contained more ash, iron, alumina and calcium than those associated with hosts. The above differences were most prominent in the case of leaves.

Other conditions being alike for the two sets of plants, it may be inferred from the above that better development of sandal raised in association with the host is due to the nutrition supplied by the latter, particularly nitrogen, phosphorus and potash. On the other hand, the values obtained for iron, alumina and calcium would point to those constituents being taken directly from the soil : the lower percentages observed in the case of plants with hosts may be largely

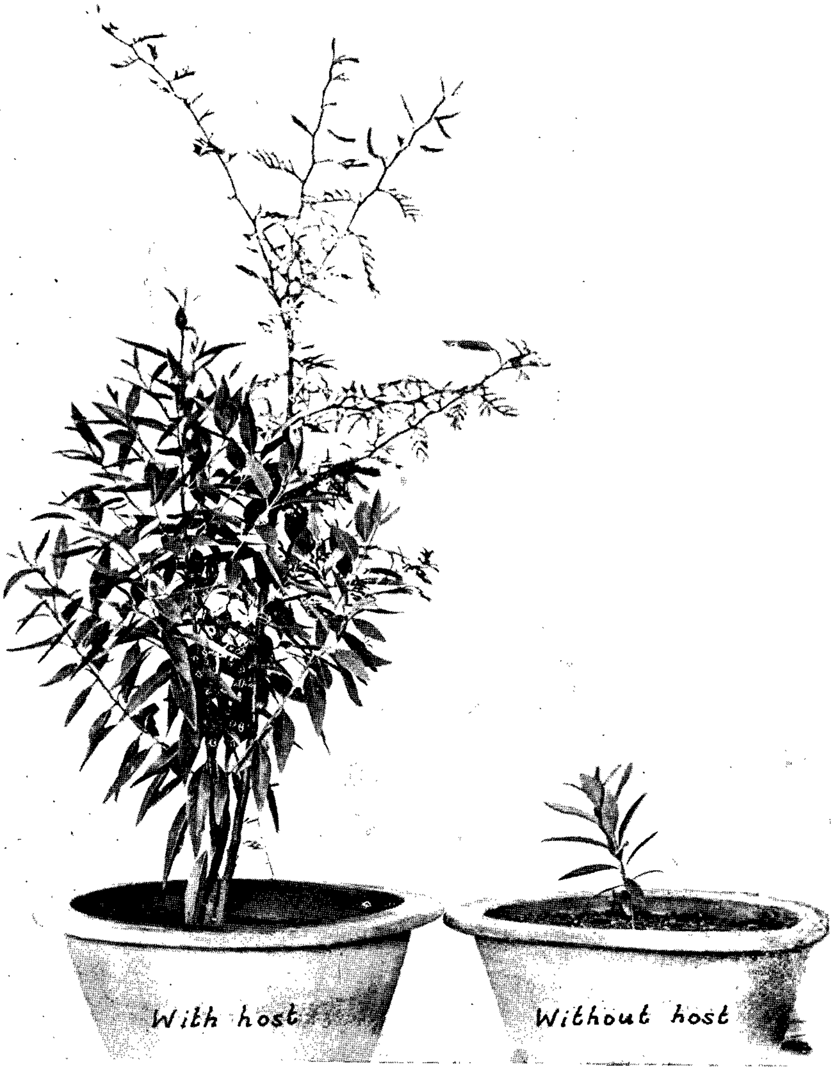

With host
Without host

TABLE I

PERCENTAGES ON MOISTURE-FREE MATERIAL

	Leaves				Stems				Roots			
	I		II		I		II		I		II	
	With host	Without host	With host	Without host	With host	Without host	With host	Without host	With host	Without host	With host	Without host
Ash	9·08 ...	13·76	8·22	14·68	3·40	7·22	3·21	7·49	3·09	5·86	3·01	6·29
Total N_2	4·22 ...	1·75	2·69	1·19	1·27	0·98	1·30	0·88	1·18	0·96	1·06	0·99
P_2O_5	0·47 ...	0·26	0·41	0·32	0·23	0·21	0·25	0·20	0·34	0·24	0·25	0·19
Fe_2O_3 and Al_2O_3	0·30 ...	1·69	0·25	0·70	0·23	0·32	0·30	0·65	0·42	0·78	0·27	0·98
CaO	1·32 ...	4·15	1·36	4·23	0·82	2·41	0·69	2·64	0·24	0·36	0·16	0·31
K_2O	3·49 ...	1·69	2·43	1·38	1·68	0·95	0·94	0·78	1·81	1·03	0·93	0·88

explained by the fact that those plants had greatly increased in total weight (made up chiefly of products of photosynthesis) as compared with the controls which made no appreciable growth.

Physico-chemical relationship of sandal and its associated host plant.—In recent years the physico-chemical study of tissue fluids has been receiving an increasing amount of attention. Study of the osmotic concentrations in auto-tropic plants by Dixon and Atkins (*Sci. Proc., Royal Dublin Soc.*, 1912, **13**, 219; 1915, **14**, 374; *ibid.*, **15**, 51) helped to throw fresh light on the problem of the ascent of sap. Particular attention has been paid by them to the effects of environment on osmotic pressure and hydrogen-ion concentration. Study of the tissue fluids has also been extended to plant diseases by various workers and a considerable amount of work is being carried out in this laboratory by Sreenivasaya and his collaborators (*Indian Inst. Sci.*, 1928, **11A**, 23).

The exchange of water between the living cell and its environment depends on the osmotic pressure of the cell. It is this pressure that regulates the flow of vital fluids from one part of a tree to another and serves the entire plant with nutrition. The determination of the osmotic pressure of the host and parasite has thrown considerable light on the mechanism of the supply of nutrition from the host to the parasite (Harris *et al*, 1914-24). In this laboratory Krishna and Ranga Rao (unpublished work) have also carried out systematic studies on the physico-chemical changes in the tissue fluids of free and attacked lac hosts.

In view of the importance of physico-chemical measurements in determining the relationship between the host and the parasite, some studies were carried out on the tissue fluids of sandal in association with different host plants.

Material.—The samples were all taken from the nursery attached to the department.

Various methods have been proposed for the extraction of plant tissue fluids, but the observations of Dixon and Atkins (*Proc. Roy. Dublin Soc.*, 1913, **13**, (N.S.) 422), and Gortner and his co-workers, (*Plant World*, 1914, **17**, 53; *Biochem. Bulletin*, 1916, **5**, 139) have shown that preliminary freezing of the tissues with either liquid air or solid carbon dioxide render the cell walls permeable so that subsequent pressing brings out all the available tissue fluid. Since our experience in this laboratory also pointed to the same conclusion, the tissue fluids investigated in the present study were obtained by first freezing the material with liquid air and then pressing it out under conditions which were standardised for each material. Thus, leaf tissues were pressed

at one ton per sq. inch for 3 minutes, roots at $1\frac{1}{2}$ tons for 5 mins., and stems at 2 tons for 7 mins. By adhering to this formula fairly uniform specimens of sap were obtained for all the experiments.

Sandal plants with hosts were carefully pulled out and washed free from adhering soil. They were then divided into three portions, stems, leaves and roots as before and the tissue fluids pressed out. The products thus obtained were centrifuged at 3,000 revolutions per minute to free them from suspended impurities. The clear sap was used for subsequent analyses.

Nitrogen and the ash constituents were determined according to the methods already referred to with the difference that when the quantities of specimens were small, the estimation of nitrogen was carried out according to the micro-method of Pregl. Osmotic pressure, electrical conductivity and hydrogen-ion concentration were determined according to the methods described in an earlier communication (*Jour. Indian Inst. Sci.*, 1928, **11A**, 104-106). The results have been given in the following tables.

It will be seen from the above that the values for the osmotic pressure of the tissue fluids from different parts of sandal are invariably higher than the corresponding quantities for the associated hosts. This observation is in conformity with those of Lawrence and Harris (*Amer. Jour. Bot.*, 1916, **3**, 438-455) on the Jamaican *Loranthaceae* and their various hosts. On the other hand, the osmotic pressures of the tissue fluids from different parts of the hosts growing by themselves under similar conditions (*vide* Tables III and V) are consistently higher than those of similar plants serving as hosts; in the case of *Pongamia glabra,* however, the values are higher than those of the associated sandal plants themselves. This is a rather striking observation as it would appear to contradict the prevalent opinion that a higher initial osmotic pressure of the parasite is necessary to cause the flow of the nutrients from the host. The results do in fact show that the diminution in the osmotic pressure of the tissue fluids of both *Acacia farnesiana* and *Pongamia glabra* is the result of parasitisation by sandal so that the flow of sap from the host to sandal would appear to be the result of some other specific attribute of the latter rather than to its tissue fluid possessing a higher osmotic pressure than that of the host. Further work on this aspect of the problem is in progress and it is expected that the results would lead to findings of fundamental importance in the study of factors relating to the nature of parasitism in plants.

The hydrogen-ion concentration of the tissue fluid from different parts of sandal is generally higher than that of the corresponding hosts.

TABLE II
Sandal-*Acacia* combination

	Percentage of sap on fresh weight	Expressed as grams in 100 c. c. of tissue fluid						Osmotic pressure in atmos.	Specific electrical conductivity × 10^5	P_H
		Total solids	Ash	Total nitrogen	P_2O_5	CaO	K_2O			
		1st Set. *Specimens collected on 23rd September 1929*								
PARASITE										
Sandal leaves ...	32·84	17·76	3·15	0·37	0·16	0·48	0·82	19·66	14,72	...
,, stems ...	25·54	12·50	1·25	0·25	0·14	0·11	0·29	12·66	8,99	...
,, roots ...	35·69	10·48	1·07	0·15	0·09	0·08	0·34	10·37	9,70	...
HOST										
Acacia farnesiana leaves	41·19	12·38	1·66	0·31	0·10	0·21	0·47	13·75	9,32	...
,, stems	21·75	9·89	1·42	0·23	0·11	0·08	0·22	10·61	9,29	...
,, roots	29·41	6·43	1·22	0·12	0·09	0·10	0·29	7·89	9,65	...
		2nd Set. *Specimens collected on 12th November 1929*								
PARASITE										
Sandal leaves ...	33·22	20·43	3·19	0·34	0·19	0·59	1·03	19·05	13,03	4·95
,, stems ...	19·56	15·02	1·35	0·21	0·17	0·33	0·49	14·34	8,38	4·90
,, roots ...	24·58	14·68	1·14	0·19	0·11	0·27	0·42	12·54	8,03	5·35
HOST										
Acacia farnesiana leaves	47·47	11·15	1·50	0·31	0·11	0·13	0·53	12·66	9,72	5·30
,, stems	19·68	8·62	1·43	0·19	0·11	0·14	0·50	9·77	8,99	4·95
,, roots	28·07	4·17	0·88	0·12	0·08	0·12	0·14	5·31	8,34	5·55

TABLE III

Acacia farnesiana alone

	Percentage of sap on fresh weight	EXPRESSED AS GRAMS IN 100 C.C. OF TISSUE FLUID						Osmotic pressure in atmos.	Specific electrical conductivity × 10^5	P_H
		Total solids	Ash	Total nitrogen	P_2O_5	CaO	K_2O			
		1ST SET. *Specimens collected on 16th September 1929*								
Leaves ...	27·60	15·07	2·03	0·39	0·12	0·27	0·67	18·76	11,05	5·10
Stems ...	20·00	8·94	1·44	0·29	0·18	0·06	0·47	12·27	10,40	5·20
Roots ...	21·30	8·28	1·36	0·26	0·11	0·08	0·45	12·27	13,10	5·65
		2ND SET. *Specimens collected on 31st October 1929*								
Leaves ...	...	11·06	1·52	0·34	0·13	0·20	0·66	16·36	9,52	5·05
Stems ...	...	8·84	1·24	0·22	0·16	0·07	0·40	14·44	9,19	4·95
Roots ...	...	7·04	1·03	0·23	0·11	0·08	0·44	10·11	9,15	5·94

TABLE IV

Sandal-*Pongamia glabra* combination

	Percentage of sap on fresh weight	Expressed as grams in 100 c.c. of tissue fluid: Total solids	Ash	Total nitrogen	P_2O_5	CaO	K_2O	Osmotic pressure in atmos.	Specific electrical conductivity × 10^5	P_H
		1st Set. *Specimens collected on 27th November 1929*								
PARASITE										
Sandal leaves ...	35·92	18·91	2·42	0·38	0·07	0·38	0·76	23·99	12,43	4·96
,, stems ...	10·91	13·26	1·36	0·22	0·11	0·08	0·49	13·83	14,55	5·05
,, roots ...	20·41	12·99	1·19	0·17	0·06	0·10	0·39	12·42	8,75	5·40
HOST										
Pongamia glabra leaves ...	14·77	19·58	2·54	0·38	0·13	0·36	0·89	16·64	9,42	5·30
,, stems ...	25·65	15·09	1·91	0·41	0·20	0·19	0·49	13·93	9,62	5·70
,, roots ...	30·87	9·32	1·56	0·39	0·06	0·07	0·84	9·53	10,84	5·55
		2nd Set. *Specimens collected on 20th January 1930*								
PARASITE										
Sandal leaves ...	39·66	19·70	3·04	0·37	0·09	0·49	...	21·16	14,18	5·18
,, stems ...	12·90	12·61	0·84	0·22	0·08	0·11	...	12·72	8,77	5·05
,, roots ...	17·13	12·26	0·88	0·16	0·06	0·11	...	10.55	9,40	5·50
HOST										
Pongamia glabra leaves ...	28·15	9·35	1·15	0·40	0·09	0·26	...	17·59	9,68	5·33
,, stems ...	29·41	10·31	1·49	0·70	0·11	0·20	...	10·91	12,46	5·70
,, roots ...	32·43	8·39	1·57	0·46	0·06	0·11	...	9·17	15,10	5·98

TABLE V

Pongamia glabra alone

Specimens collected on 11th February 1930

	Percentages of sap on fresh weight	Expressed as grams in 100 c.c. of tissue fluid					Osmotic pressure in atmos.	Specific electrical conductivity × 10^5	P_H
		Total solids	Ash	Total nitrogen	P_2O_5	CaO			
Leaves	43·93	5·47	0·89	0·41	0·16	0·08	25·31	11,18	5·80
Stems	42·31	8·73	1·20	0·75	0·22	0·15	19·11	9,67	5·50
Roots	47·82	8·69	1·40	0·68	0·08	0·11	14·97	7,67	5·80

The tissue fluid from the leaf would appear to be slightly more acid than that from the roots. The values of specific electrical conductivity throw no light on the nature of the relation between the host and the parasite. The conductivity of the sap generally decreases as we pass from the leaf to the root. A similar observation would also apply to the total solids, ash, nitrogen, calcium and phosphorus.

In view of the dependence of sandal on its host for the supply of nitrogen and certain essential mineral constituents, some experiments were carried out to determine the effect of association of sandal with various hosts on the chemical composition and hydrogen-ion concentration of its tissue fluid. The results have been presented in the following table (Table VI).—

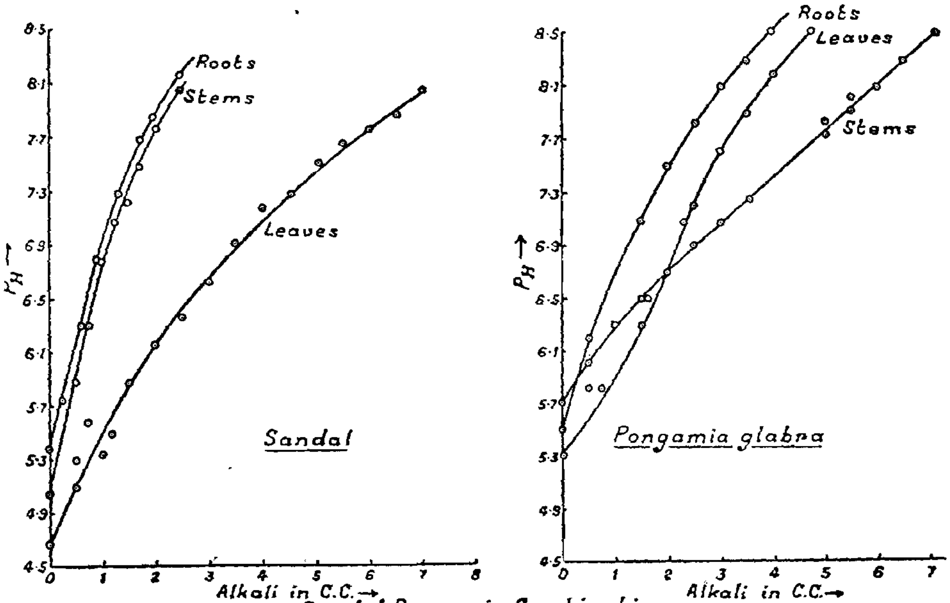
TABLE VI

Chemical composition of the tissue fluid of sandal raised in association with different host plants

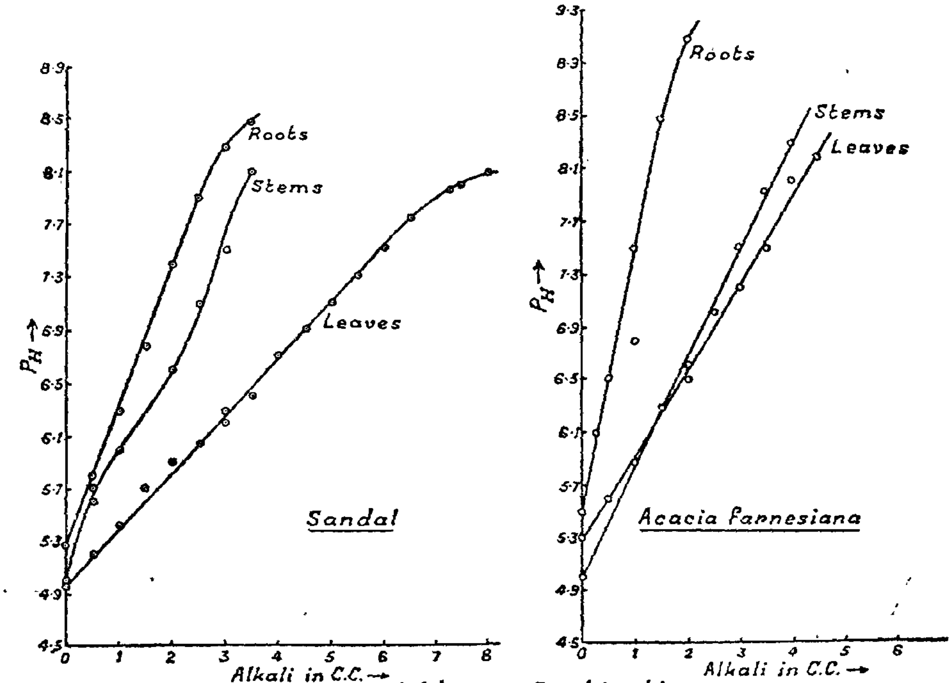
Host	In 100 c.c. of tissue fluid expressed as grams					
	Ash	Total solids	Total nitrogen	P_2O_5	CaO	P_H
Melia indica	2·83	15·02	0·33	0·09	0·67	5·6
Lantana camara	3·96	13·47	0·08	0·08	0·09	4·8
Acacia farnesiana ...	3·15	17·76	0·37	0·16	0·48	5·0
Pongamia glabra	2·42	18·91	0·38	0·08	0·38	5·3
Casuarina aquisetifolia ...	1·03	14·81	0·28	0·05	0·35	5·1
Dendrocalamus strictus ...	0·83	12·43	?	0·07	0·18	5·6–5·8

It may be seen from the above that the chemical composition of the tissue fluid of sandal is largely determined by the nature of the associated host. Taking nitrogen, for instance, it may be seen that the plants in association with the leguminous hosts have generally larger quantities of that element than those parasitising on other hosts. On the other hand, legumes render sandal susceptible to spike-disease while several other classes of plants are known to impart resistance. The significance of nutrition in relation to resistance to disease is an important aspect of the physiology of sandal and will form the subject of a later study.

The above observations are also of great importance in relation to the sylviculture of sandal. The introduction of the right kind of host



Sandal-Pongamia Combination



Sandal-Acacia Combination

not only enriches the tissue fluid of sandal but also promotes vigorous growth and in some cases imparts resistance to disease. The preponderance of comparatively poor hosts or lack of sufficient number of good hosts impoverishes the tissue fluid, impairs the growth and renders the plant susceptible to disease.

Buffering capacities of the tissue fluids of sandal and its associated hosts.—This was determined by titrating the tissue fluids against alkali and determining the quantities of the latter required for each unit shift of the hydrogen-ion concentration. The results presented in graphs I to IV show that the tissue fluid from different parts of sandal has higher buffering capacity than those from corresponding parts of the hosts. The different parts of sandal in association with *Acacia farnesiana* have higher buffering capacity than those of plants parasitising on *Pongamia glabra*. The possible relation of this observation to the greater susceptibility to spike observed in the case of sandal associated with *Pongamia* is under investigation.

SUMMARY.

1. Sandal depends on its host for its requirements of nitrogen, phosphorus and potash : the other ash constituents including calcium, iron and alumina would appear to be directly derived from the soil.

2. The tissue fluids from different parts of sandal have consistently higher osmotic pressure than those derived from corresponding parts of the associated hosts. The higher osmotic pressure observed in the case of the hosts (particularly *Pongamia glabra*) when growing alone would suggest that the lower values obtained in the previous case are the result of parasitisation by sandal and not a cause as generally believed.

The hydrogen-ion concentration of sandal is higher than those of the two hosts which were investigated. The buffering capacity of the tissue fluid of sandal in association with *Acacia farnesiana* is slightly higher than that of sandal with *Pongamia glabra* as host. The chemical composition of the tissue fluid of sandal is dependent upon the nature of the host with which it is associated.

My thanks are due to Mr. M. Sreenivasaya for many useful suggestions and to Prof. V. Subrahmanyan for his keen interest in the work.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF SANDAL

PART II. Influence of host on the nitrogen metabolism of sandal.

By Y. V. Sreenivasa Rao.

The study of nitrogen metabolism of plants is of very great importance in view of its intimate relationship with the fundamental activities of life such as growth and reproduction. The metabolic changes proceed in the different parts of the plant but those occurring in the leaf are of special interest since it is the seat of protein synthesis and other related activities. Sandal being a root parasite, the changes taking place in its system would throw light on the nature of the nitrogenous constituents which the plant is capable of synthesising and the extent to which it depends on the host for elaborated food. Since the haustorial connections of sandal die out with the onset of spike-disease, it appeared probable that the conditions in diseased sandal would at least partly correspond to those in sandal when it is deprived of its host. In view of the above and the observation of Sreenivasaya that association with certain plants render sandal susceptible to spike while that with others imparts resistance to artificial infection of the disease, it was considered that the study of nitrogen metabolism of sandal, with and without hosts, would lead to findings of some practical importance.

EXPERIMENTAL.

The experimental plants were raised and the leaf materials prepared in the manner described in the previous part (*Jour. Indian Inst. Sci.*, 1933, **16A**).

Methods.—Total nitrogen was determined according to Gunning's modification of Kjeldahl's method to include nitrates. Protein nitrogen was estimated by the Stutzer's method (*J. Land.*, 1880, **28**, 103). Nitrates were estimated according to the method of Scales (*J. Biol. Chem.*, 1916, **27**, 327) and nitrite by colorimetric method using *Griess* Ilosway reagent for the purpose. Ammonia was estimated according to Grafe and Erich (*Zeit. Physiol. Chem.*, 1906, **48**, 300). Distribution of water-soluble nitrogen was determined by the methods of Jodidi, Kellog and True (*Jour. Agric. Res.*, 1918, **15**, 385) and amino nitrogen by the Van Slyke method (*Jour. Biol. Chem.*, 1911, **9**, 185 ; *ibid.*, 1912, **12**, 275). The details of the methods as adopted for

different determinations are similar to those described by Narasimhamurthy and Sreenivasaya in their investigation on the distribution of nitrogen in healthy and spiked sandal (*Jour. Indian Inst. Sci.*, 1929, **12A**, 153).

As mentioned in a previous paper it was observed that sandal in association with the host (*Acacia farnesiana*) had a higher percentage of nitrogen (2.69 to 2.84) than the one without the host (2.03 to 2.19). Nitrite could not be detected in the extracts from sandal growing in association with the hosts while it was present only in traces in the control set of plants. Quantitative determination of nitrites was not therefore carried out.

The other results have been presented in the following tables :—

TABLE I

Distribution of nitrogen in the leaves of sandal with and without host plant.

Form of Nitrogen	Percentages on moisture-free basis			
	Set I		Set II	
	With host	Without host	With host	Without host
Total nitrogen ...	2·83	2·03	2·84	2·05
Protein ,, ...	2·07	0·86	2·08	0·86
Nitrate ,, ...	0·07	0·06	0·07	0·06
Total water-soluble N ...	0·68	1·19	0·77	1·20
Ammonia N ...	0·01	0·04	0·01	0·05
Amide ,, ...	0·08	0·01	0·06	0·01
Humin ,, ...	0·09	0·20	0·12	0·20
Basic ,, ...	0·14	0·46	0·19	0·44
Mono-basic ,, ...	0·19	0·21	0·19	0·21
Non-basic ,, ...	0·13	0·25	0·18	0·25
Peptide ,, ...	0·004	0·00	0·00	0·00

TABLE II

Distribution of the water-soluble nitrogen in the leaves of sandal with and without host plants

Material	Percentage of HCl used	Time of digestion in hours	Percentage of the total water-soluble nitrogen						
			Ammonia	Amide	Humin	Basic	Mono-amino	Residual	Peptide
With host	4	½	1·89	11·76	13·60	20·29	28·11	18·94	...
Do.	20	8	1·89	11·76	13·60	...	30·01	17·04	0·63
Without host ...	4	½	3·75	0·50	13·49	38·87	17·72	21·16	...
Do.	20	8	3·75	0·50	13·49	...	21·30	17·58	...
With host	4	½	1·67	7·91	16·20	24·06	24·02	22·94	...
Do.	20	8	1·67	7·91	16·99	...	26·04	20·92	0·49
Without host ...	4	½	3·73	0·56	16·63	36·90	17·61	21·01	...
Do. ...	20	8	3·73	0·56	16·63	...	20·30	18·30	...

TABLE III

Percentages of nitrogen in the leaves of sandal occurring in association with different hosts

Host	Nitrogen	Host	Nitrogen
antana camara ...	1·29	*Murraya koenigii* ...	2·77
ivi Divi	2·17	*Dendrocalamus strictue* ...	1·58
asuarina aquisetifolia ...	2·91	*Pongamia glabra* ...	3·23
Melia indica	2·78	*Acacia farnesiana* ...	4·22
Rutea graveolens	2·79		

TABLE IV

Form of Nitrogen	Results expressed as per cent. of total N.	
	With host	Without host
Total basic nitrogen ...	5·26	22·87
Amino N.	2·45	8·64
Non-amino N. ...	2·81	14·23
Arginine N. ...	3·38	17·07
Histidine N.	0·49	2·14
Cystine N.	0·41	0·42
Lysine N.	1·01	3·23
Nitrogen in filtrate from bases	13·40	23·06
Amino N.	8·88	12·50
Non-amino	4·52	10·56

It may be seen from the above that the nitrogen content of sandal is determined by the nature of the host with which it is associated. Thus, the plants growing in association with the two leguminous hosts, *Pongamia glabra* and *Acacia farnesiana,* have much higher nitrogen contents than those with other hosts. The results, together with pot culture observations, showed that the supply of suitable hosts

particularly those of leguminous type is necessary to promote the rapid vegetative growth of sandal. Sandal without hosts leads a struggling existence and since the improved growth observation in association with a host is accompanied by increase in nitrogen content, it would appear that the supply of nitrogen by the host plant is one of the limiting factors in the growth of sandal, at any rate, in the early stages. The leaves of sandal without host turn yellow and the plants make no appreciable growth but if the host plant is provided, preferably one of leguminous type, the natural green colour is restored and the plant makes very rapid growth.

The nitrate content of sandal with host is slightly higher than that of the one without it. Sandal fed by host is richer in amide nitrogen than the one without it. Since amides play a very important part in transformations of nitrogen into proteins it would appear the conditions are more favourable in sandal with host than in the one without. This observation is supported by the protein contents which show that the host-fed sandal is richer than the one without (Table I). The observations relating to synthesis of proteins are further supported by the comparatively smaller amounts of water-soluble nitrogen in host-fed plants while the corresponding condition in hostless sandal would point to proteolytic degradation taking place therein.

The state of sandal that is deprived of its host corresponds to that of starvation observed by Chibnall in the case of the runner bean plant which showed decrease in protein nitrogen, followed by increase in water-soluble forms with the progress of starvation (*Biochem. J.*, 1912, **16**, 344). Chibnall also observed that the starved plants contained a greater quantity of what he called the 'other N', which corresponds to the 'residual N' in column 10 of Table II in the present paper. Drastic treatment (with concentrated HCl for 16 hours) to effect complete hydrolysis led to increase in ammonia and basic nitrogen followed by decrease in 'other N'. This suggested to Chibnall that 'other N' might consist of ureides like barbituric acid which resist mild hydrolysis, but which, on prolonged hydrolysis, yield bases giving a precipitate with phosphotungstic acid. Basing his conclusions on those of Fosse (*Compt. rend.*, 1913, **156**, 567, 1938; 1914, **158**, 1374) who demonstrated the presence of traces of urea in higher plants, Chibnall drew the conclusion that the catabolic process of the nitrogen metabolism in the leaves proceed along the line leading to increased production of 'other N'. The results presented in column 10 of Table II are similar to those obtained by Chibnall. Although drastic hydrolysis with concentrated acid was not conducted, yet prolonged treatment with 20 per cent. hydrochloric acid led to increase in the mono-amino nitrogen content of the hydrolysate, thereby suggesting that the latter was derived from the 'residual' nitrogen. It would follow therefore

that the values for mono-amino nitrogen given in column 9 of Table II, did not represent the entire amount of mono-amino nitrogen present in the water extract. Another reason for concluding that column 9 does not represent the actual amount of mono-amino nitrogen is that according to Hart and Bently (*J. Biol. Chem.*, 1915, **22**, 477) and Roxas (*J. Biol. Chem.*, 1916, **23**, 71) at least a part of the humin nitrogen formed as a result of hydrolysis with HCl is formed out of the mono-amino nitrogen. For the same reason column 11 cannot represent the actual amount of peptide nitrogen present in the extract as part of the increase of amino-nitrogen due to hydrolysis of peptide by acid may be converted into humin nitrogen. In view of these defects in the method of acid hydrolysis, a development of the method of hydrolysing the peptides with a specific enzyme seems necessary for the estimation of the true peptide nitrogen.

The results given in Table III show that the nitrogen contents of sandal leaves in association with different host plants are dependent on the nature of the hosts with which it is associated.

Table IV gives the results of the distribution of the hexone bases in the leaves of sandal with and without host plant according to the method of Van Slyke as modified by Plimmer and his co-workers (*Biochem. J.*, 1925, **19**, 1004; *ibid.*, 1927, **21**, 247). The proportion of water-soluble nitrogen in the leaves of sandal with and without host is as 1 :3 and the amino nitrogen in the filtrate obtained after separation of bases is less in the case of sandal with host plant than in one without. The significance of these observations is still somewhat obscure.

When the hexone bases are expressed as percentages of the total basic nitrogen arginine and histidine are found to exist to the same extent in sandal raised with and without hosts. Lysine is present to a slightly greater extent and cystine very much more so in sandal nourished by hosts than in without. Since cystine is known to be essential for the growth and active functioning of plants, it is probable that the inadequacy of supply of that essential amino-acid may be partly responsible for the poor development of hostless sandal.

SUMMARY

A detailed investigation of the nitrogen metabolism in the leaves of sandal with and without hosts has been made.

Total nitrogen in the leaves of sandal is largely influenced by the nature of the host plant with which it is associated.

A great proportion of the nitrogen in host-fed sandal exists in the form of protein. Absence of host plant seems to bring about a

decrease in protein followed by an increase in the amount of water-soluble nitrogen.

Basic nitrogen content of leaves of hostless sandal is greater than that of the host-fed plant.

Sandal without host resembles the spiked plant in that its protein nitrogen is low and water-soluble nitrogen (made up chiefly of basic forms) high. On the other hand, the ammonia content of hostless sandal is higher and amide nitrogen lower than that of host-fed sandal. This observation differs from that in the case of spiked sandal, the ammonia and amide contents of which are not significantly different from those of the healthy one.

In conclusion, my thanks are due to Mr. M. Sreenivasaya for many useful suggestions and to Prof. V. Subrahmanyan for his keen interest in the work.

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INDIAN COAL TAR

By S. K. Ganguly, B. Sanjiva Rao and P. C. Guha.

The possibility of the development of the coal tar industry in India is remote, as the quantity of tar available is small, only two fair-sized gas works, at Bombay and Calcutta, being in operation. Coal of good quality is found only in the Gondowana fields of Behar and Bengal, while the deposits in the adjacent areas of Central India consist of an inferior coal characterised by high ash and low calorific value. Coal of tertiary age generally inferior in quality is also found in Bikanir, the Punjab and Baluchistan. This low grade coal is not suitable for metallurgical purposes and only the best varieties are treated in coke ovens. Very little information is available regarding the tar which is at present manufactured and as it appeared that a comparison with material from different sources would be of interest, it was decided to examine any available samples and determine their chief constituents.

The two main sources of coal tar, *viz.*, gas tar and coke oven tar, in the West are also produced in India and specimens were obtained from the Oriental Gas Company, Calcutta, the Bombay Gas House, and the Jamshedpur coke ovens. The coal from which these were produced was obtained from the Raniganj coalfield, Dhemo Main and Parbelia, and the Jharia coalfield respectively. Coal tar being a product of destructive distillation, its composition varies with the varieties of coal used, plant and details of distillation and there are marked differences between the tar obtained in the manufacture of coal gas and that collected at the coke ovens. In gas works, vertical retorts which are gradually displacing the horizontal, give a tar having a lower specific gravity, less free carbon and pitch and more of tar oils. The gas tars examined are of normal quality and comparable with tars produced in a similar way in Europe the coke oven tar from Jamshedpur being an exception.

The first treatment of coal tar on a large scale consists in distilling it and collecting the distillate in four or five fractions. A few distillations with 25 lb. charges in a small iron still designed for the purpose were made but as larger quantities of some fractions were required, it was found more convenient to employ the fractions obtained by the Bengal Chemical and Pharmaceutical Works from the Oriental Company's gas tar.

EXPERIMENTAL

The two specimens of tars from the gas works were obtained in 20 gallon drums while that from Jamshedpur in a large barrel containing 500 lbs. They were thoroughly mixed in the containers and 3 litre samples withdrawn from each and kept in wide mouthed bottles.

Estimation of water.—The tar (100 g.) was mixed with benzene (50 c.c.) in a glass retort (250 c.c.) and distilled until a temperature of 200° was reached. The volume of water which separated was read off from the measuring cylinder into which the distillate had been received (Atack, *The Chemists' Year Book*, 1932, p. 982). This determination was also made by directly distilling the tar and the volume of water passing over with the distillate up to 170° was collected and measured.

Specific gravity.—The tar was dehydrated before the specific gravity was determined as follows. An open beaker containing the tar was placed in a water-bath kept at 50° for 24 hours. The water which separated was decanted and the last traces removed with filter paper. The specific gravity of the dehydrated tar was determined using a specific gravity bottle (50 c.c.) with a vertical groove in its stopper (Illingworth, *The Analysis of Coal and its By-products*, 1921, p. 139).

Free carbon.—The tar (10 g.) was introduced into a fat-free thimble (previously washed with xylene and dried and weighed) and extracted with xylene in a soxhlet apparatus until the solvent flowed out clear and no further change occurred in the weight of the thimble after drying. The weight of the free carbon in the thimble was determined and the percentage calculated.

Ash.—The tar (5 g.) was weighed in a porcelain crucible provided with a lid, heated cautiously to expel volatile matter and then ignited, cooled and weighed (*Standard Methods for testing Tar and its products.* By 'Standardisation of Tar Products Tests Committee'. W. Heffer & Sons, 1929, p. 41).

Distillation test.—The procedure and the conditions suggested by Illingworth (*ibid.*, pp. 133-137) for the distillation of the tar were adhered to throughout, as a slight change in the rate of distillation or the variation of half-an-inch in the position of the thermometer causes a difference of about 5 per cent. in the amount of distillate (*ibid.*, p. 135). The proportion of the various fractions is a rough estimate of the quantities of several products obtainable in works practice. The temperatures at which receivers are changed, differ considerably in the practice of different analysts and the following has been adopted in this

TABLE I.

Analysis of Coal tars.

—	Gas tar from Oriental Gas Co., Calcutta	Gas tar from Bombay Gas House	Coke oven tar from Jamshedpur	Gas tar horizontal retort	Gas tar vertical retort	Coke oven tar
$d^{15\cdot5}_{15\cdot5}$ ...	1·1720	1·1408	1·2445	1·212	1·103	1·142
Water	2·0	4·0	...	3·9	3·8	2·0
Free carbon in tar ...	9·4	2·6	17·4	22·0	2·4	4·0
Ash	0·07	0·12	0·07	...	...	...
Light oil (–170°) ...	3·2	8·2	*Nil*	3·4	9·1	4·1
Carbolic oil (170–230°) ...	12·5	12·5	*Nil*	6·0	10·2	8·0
Creosote oil (230–270°) ...	10·8	10·4	8·2	8·0	11·2	16·2
Anthracene oil (about 270°).	8·2	10·2	10·8	15·1	16·6	18·7
Pitch	62·8	54·2	80·0	67·5	51·9	53·0

investigation, figures in brackets giving the percentage of each fraction obtained from Calcutta gas tar: ammonia liquor, -110° (2-0); light oil, 110-170° (2-3); carbolic oil, 170-230° (8-10); creosote oil, 230-270° (8-10); anthracene oil (light), 270-350° (10); anthracene oil (heavy), above 350° (5); pitch, residue (62-65). The results obtained during the analysis of the several tars are given in Table I along with the constants for some typical gas and coke oven tars for comparison.

Tars are liable to vary from many causes and the results given for comparison in the table must therefore be looked upon only as a guide. The gas tars from Calcutta and Bombay compare well with vertical retort gas tars produced in other countries. Coke oven tar from Jamshedpur does not contain any of the lower boiling hydrocarbons or the lower phenols. The product does not compare well with those obtained from any of the different kinds of coke ovens.

The information obtained so far gives a general basis for the evaluation of the crude tars. Further examination is now necessary to determine the quantity of refined products obtained. The gas tar from Calcutta alone has been further examined. The tar (25 lb.) was distilled from a 5 gallon iron still designed for the purpose with a convex bottom and a stop-cock for running off the molten pitch, using a Fletcher burner during the distillation.

Larger quantities of the various fractions of the Calcutta gas tar were made available to us by the Bengal Chemical and Pharmaceutical Works to whom our best thanks are due for thus facilitating the work.

Light Oil

The light oil fraction formed 2.5 per cent. of the whole tar, had $d\,^{15.5}_{15.5}$ 0.9971 and contained 0.5 per cent. water. The crude light oil (1030 g.) on cooling gave naphthalene (85.5 g.) or 0.2 per cent. on the whole tar. The light oil was analysed for its constituents according to the methods of Colman and Yeoman (*J. Soc. Chem. Ind.*, 1919, **38**, 567).

Phenols and bases.—The crude light oil (600 c.c.) was fractionated twice using a 12-pear Young column and 387 c.c. (65 per cent. crude light oil or 1.63 per cent. of the whole tar) distilled over below 200°. This fraction is hereafter called the prepared light oil. The prepared light oil (150 c.c.) was shaken with 10 per cent. aqueous caustic soda (40 c.c.) for five minutes and allowed to settle for half-an-hour. It was washed again with caustic soda. The loss of volume (31 c.c. or 20.7 per cent by volume on prepared light oil or 0.3 per cent. on the whole tar) represents the phenols or tar acids present.

The tar (119 c.c.) freed of the phenolic bodies was shaken with three separate volumes of 25 c.c. sulphuric acid (1 :2) (Illingworth, *ibid.*, p. 206) the acid extracts mixed, washed with ether and basified with 20 per cent. caustic soda solution. The liberated bases measured 14 c.c. or 0.15 per cent. on the whole tar or 9.3 per cent. on the prepared light oil.

Unsaturated hydrocarbons.—The tar devoid of phenols and bases was shaken with 95 per cent. sulphuric acid (13 c.c.) for exactly 5 minutes and a diminution of 18 c.c. (0.2 per cent. on whole tar or 12 per cent. on prepared light oil) was observed.

Determination of benzene, toluene and xylene.—Phenols, bases and unsaturated hydrocarbons having been removed, the oil (300 c.c.) was fractionated with a 12-pear Young column at the rate of 4 c.c. per minute and the following fractions were obtained :

Fraction	B. P. at 685 mm.	B. P. at 760 mm.	Vol. of oil in c.c.
A	-91·7	-95	45
B	91·7–136·5	95–140	76
C	136·5–156·3	140–160	39
D	156·3–196·3	160–200	100
E	Above 196·3	Above 200	37

'90's benzol.—On fractionating A (45 c.c.), the oil boiling upto 90° was 37 c.c. which represents 90's benzol, i.e., the fraction upto 90° (7.1 per cent. on prepared oil).

Benzene and toluene.—90's benzol (100 c.c.) on fractionation gave 84.2 c.c. boiling below 81.7° (85° at 760°). The amount of benzene read off from Colman and Yeoman's graph was 91.3 per cent. and toluene 8.7 per cent.

The fraction 90-140° (fraction B + residue from 90's benzol) gave on distillation at 685 mm. the following results :—

-101·5 (105° at 760 mm)	...	45·5 c. c.
101·5–112·2 (116° at 760 mm)	...	9·8 c. c.
Residue	...	44·2 c. c.

The benzene, toluene and xylene read off from the graph were 24, 55 and 21 per cent. respectively.

Solvent naphtha.—Fraction C (39 c.c.) along with xylene in fraction 90-140° represents solvent naphtha, i.e., 6.9 per cent. on prepared light oil or 0.17 per cent. on the whole tar.

Heavy naphtha.—The fraction 160-200° (100 c.c. or 12.5 per cent. as prepared light oil or 0.3 per cent. on the whole tar) represents heavy naphtha.

The percentage composition of the prepared light oil is as follows :—phenols, 20.7 ; pyridine bases, 9.3 ; unsaturated hydrocarbons, 12.0 ; benzene, 9.8 ; toluene, 9.2 ; solvent naphtha, 6.9 ; heavy naphtha, 12.5 ; naphthalene, 8.2.

Carbolic Oil or Middle Oil

Specific gravity d^{25}_{25} 0.9876 ; $d^{15.5}_{15.5}$ 0.9947. On distillation at 685 mm. up to 170°, 1.8 per cent. water was collected. Between 140-195° and 195-210°, 27 and 44 per cent. distilled over or 71 per cent. below 210°. According to Lunge and Keane a good quality carbolic oil should contain 60 per cent. distilling below 210°.

Naphthalene.—The oil (197.5 g.) kept at ordinary temperature (21-25°) for 24 hours deposited naphthalene (22.5 g.) or 11.4 per cent. of the carbolic oil (1.0 per cent. on whole tar).

Phenols.—The oil (400 c.c.) shaken with 20 per cent. warm sodium hydroxide (80 c.c.) showed a diminution in volume of 120 c.c. or 30 per cent. by volume. The phenols (100 c.c.) on distillation at 685 mm. yielded the following fractions :

Fraction	B. P.	Volume in c. c.	Remarks
A	170–186°	23·5	50 per cent. phenol.
B	186–195°	34·0	31 per cent. *m*-cresol.
C	195–210°	3·4	...
D	210–220°	0·6	...

According to Fox and Barker's method (*J. Soc. Chem. Ind.*, 1917, **36**, 842 ; 1920, **39**, 169) fraction *a* was found to consist of 50 per cent. phenol or 3.6 per cent. on the middle oil ; fractions *b* and *c* mixed together (10 g.) gave trinitro-*m*-cresol (5.4 g.) by Raschig's method (Illingworth, *ibid.*, p. 192) showing the *m*-cresol content to be 31 per cent. or 3.4 per cent. on the middle oil ; *o* and *p*-cresols formed 11.2 per cent. on the middle oil.

Creosote Oil

This portion of the tar boiling mainly between 230-270° had water 0.1 per cent., d^{25}_{25} 1.0244, d^{38}_{38} 1.0147 and deposited no naphthalene on cooling to 15.5° (Illingworth, *ibid.*, p. 204) ; fraction 220-230/685 mm. of the neutral oil, however, deposited 13.9 per cent. naphthalene on creosote oil. On distillation at 685 mm. 67.5 and 21.2 per cent.

distilled over between 210-250 and 250-300 respectively. The creosote oil contained phenols (13.5 per cent. by volume) and bases (3.1 per cent. by volume). The neutral oil (100 c.c. ; d $\frac{25}{25}$ 1.0156) on distillation at 685 mm. gave the following results :

Fraction	B. P.	Volume in c. c.	Remarks
1	-220°	18·0	From 1 and 2, 16.7 naphthalene
2	220-230°	16·3	...
3	230-250°	29·3	Liquid
4	250-260°	6·3	Liquid
5	260-270°	3·4	Partial solidification
6	270-320°	5·5	3·7 g. yellow solid

ANTHRACENE OIL

The anthracene oil (light) on analysis gave the following results : d $\frac{25}{25}$ 1.0373 ; phenols 13.9 per cent. ; bases (extracted with 9 per cent. hydrochloric acid) 4.2 per cent. On cooling no naphthalene or anthracene separated. The neutral oil (81 per cent.) gave 14.56 per cent. respectively between 240-270°, 270-300° and 300-340°. The last fraction partially solidified and yielded 1.6 per cent. crude anthracene or 8.9 per cent. of the anthracene oil.

Bases.—The liberated bases (246 g.) were distilled with a 8-pear Young column at 685 mm. pressure and the following fractions obtained :

Fraction	B. P.	Weight in g.	Yield per cent. in total bases
I	170-230°	14·0	5·3
II	230-260°	65·5	22·8
III	260-275°	19·8	8·0
	Residue	143·0	58·1

Isolation of quinoline and isoquinoline.—The bases (220 g.) boiling between 230-255° were obtained by fractionation of the crude bases twice. The isoquinoline which is a stronger base than quinoline was isolated from the above fraction by the method of Harris and Pope (*J. C. S.*, 1922, **121**, 1029). The fraction was shaken with sulphuric acid (4 N) for half a minute and the unattacked portion removed with benzene. The bases were then fractionally precipitated, adding enough ammonia (4 N) to the acid solution to liberate only 20 per cent. of its content in bases and extracting them with benzene every time. After eleven repetitions of the ammonia treatment, the whole of the remaining base was precipitated with excess of ammonia. The last benzene extract contained isoquinoline. The benzene is distilled and the residue mixed with twice its volume of alcohol and concentrated sulphuric acid added, keeping the temperature at 25-30°. The crystalline isoquinoline sulphate (m.p. 206° ; 1.5 g.) separated out on

standing, the yield of isoquinoline being 0.08 per cent. on the anthracene oil. From the earlier fractions quinoline (144.5 g.) was liberated or 1.3 per cent. of the anthracene oil.

Heavy anthracene oil.—This fraction boiled above 350° and had d $^{45}_{45}$ 1.0758 ; bases 2.5 per cent. (extracted with 9 per cent. hydrochloric acid) ; phenols 6.7 per cent. (with 25 per cent. aqueous caustic soda).

Anthracene.—On cooling the heavy anthracene oil (200 g.) to 10°, a solid separated, which was washed with alcohol and dried and found to be crude anthracene (18.2 g.) or 9.1 per cent. of the anthracene oil. The anthracene in this crude material was estimated by Luck's process (Illingworth, *ibid.*, p. 199). From crude anthracene (1 g.) anthraquinone (0.35 g.) was obtained or 2.7 per cent. anthracene on heavy anthracene oil.—

SUMMARY

1. The gas tars from Calcutta and Bombay gas houses are of normal quality and compare well with similar tars produced in Europe.

2. The coke oven tar from Jamshedpur was of poor quality and contained no light oil. 80 per cent. being pitch.

3. The different fractions of the gas tar from Calcutta had the following percentage composition :

(*a*) *Light oil*—Amoniacal water 0.6 ; benzene 6.8 ; toluene 7.2 ; solvent naphtha (xylene and others) 6.7 ; heavy naphtha 12.2 ; pyridine bases 5.6 ; phenol 1.3 ; cresols 14.1 ; naphthalene 6.3.

(*b*) *Middle oil.*—Phenol 3.6 ; *o* and *p*-cresols 11.2 ; *m*-cresol 3.4 ; naphthalene 11.4.

(*c*) *Creosote oil.*—Phenols 13.5 ; bases 3.1 ; naphthalene 13.9.

(*d*) *Anthracene oil.*—Quinoline 0.9 ; isoquinoline 0.03 ; anthracene 2.7.

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TAYLOR'S FREQUENCY TRIPLER.

BY

P. Rajagopal Pillai and F. N. Mowdawalla.

DR. M. O. FORSTER, F.R.S., CHAIRMAN OF EDITORIAL BOARD.

TAYLOR'S FREQUENCY TRIPLER.

By P. Rajagopal Pillai and F. N. Mowdawalla.

Taylor's frequency tripler was introduced in 1914 (*J.I.E.E.*, 1914, **54**, 701), and although it has proved to be useful as a frequency changer the principle of its action does not appear to be fully understood. The explanation of its action in Taylor's original paper (*loc. cit.*) is inadequate, but no further work on the subject has been recorded and recent books (Morecraft, *Principles of Radio Communication*, 1927, 708) explain it in practically the same terms as in Taylor's paper. The present communication aims at explaining its action more fully, removing some misconceptions and describing some experiments which serve to throw light on its action.

THEORY.

The tripler consists of three saturated choke coils, each connected in series with one of the three windings of an unsaturated transformer of which they jointly form the primary. When the arrangement is

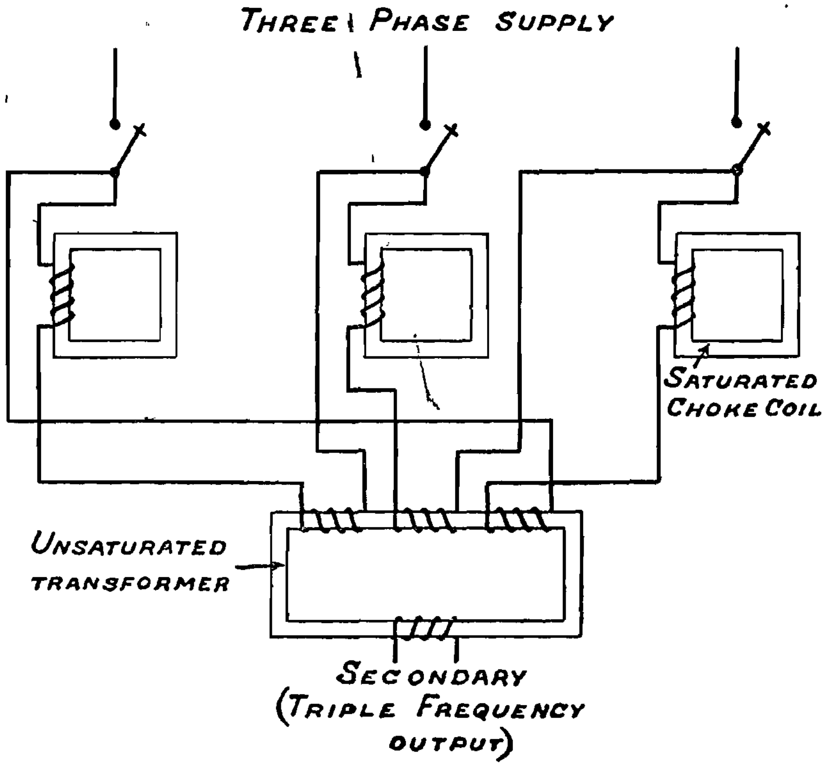


FIG. 1

DIAGRAM OF CONNECTIONS FOR TAYLOR'S FREQUENCY TRIPLER.

connected in delta and supplied with three-phase currents as shown in fig. 1, single-phase current of triple frequency can be obtained from the secondary of the transformer.

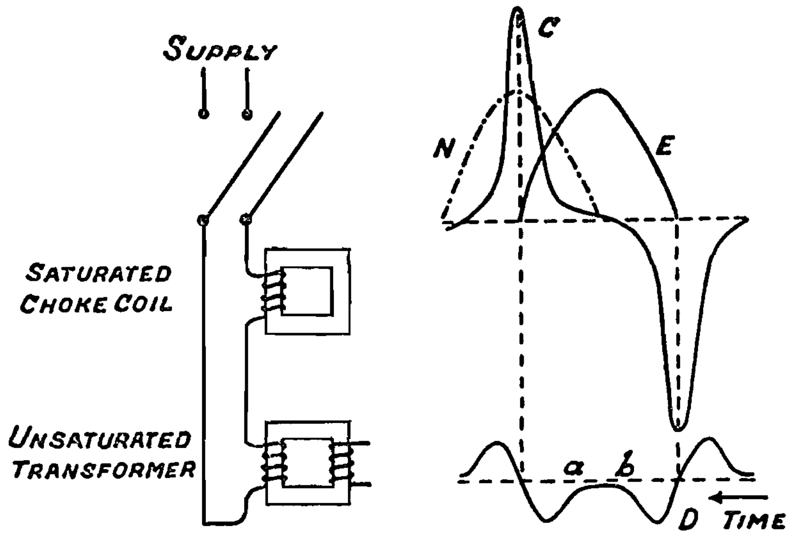


FIG. 2
PRINCIPLE OF TAYLOR'S ARRANGEMENT

Taylor's theory.—Taylor has explained the principle of the frequency changer as follows. A single-phase E.M.F., E is impressed on the saturated choke coil (fig. 2), and N represents the flux in the choke coil and C the current required to produce the flux. The current C, flowing through the unsaturated transformer produces a flux similar to itself in shape. The rate of change of this flux produces the E.M.F. represented by D, in the primary and secondary windings of the transformer. This E.M.F. wave is a symmetrical mutilated triple harmonic wave in which every third half-wave is suppressed. If three of these triple harmonic waves produced by three-phase currents are combined, the resultant is a complete triple harmonic wave as shown in fig. 3. Taylor does not give any experimental curves in support of his theory and it is not borne out by the experiments carried out by the authors.

Proposed theory.—Curve D (fig. 4) was obtained experimentally and represents the E.M.F. induced in the secondary of the unsaturated transformer when a sinusoidal P.D., V was impressed on the circuit shown in fig. 2. E is the P.D. across the terminals of the choke coil and is equal to the difference between V and D.

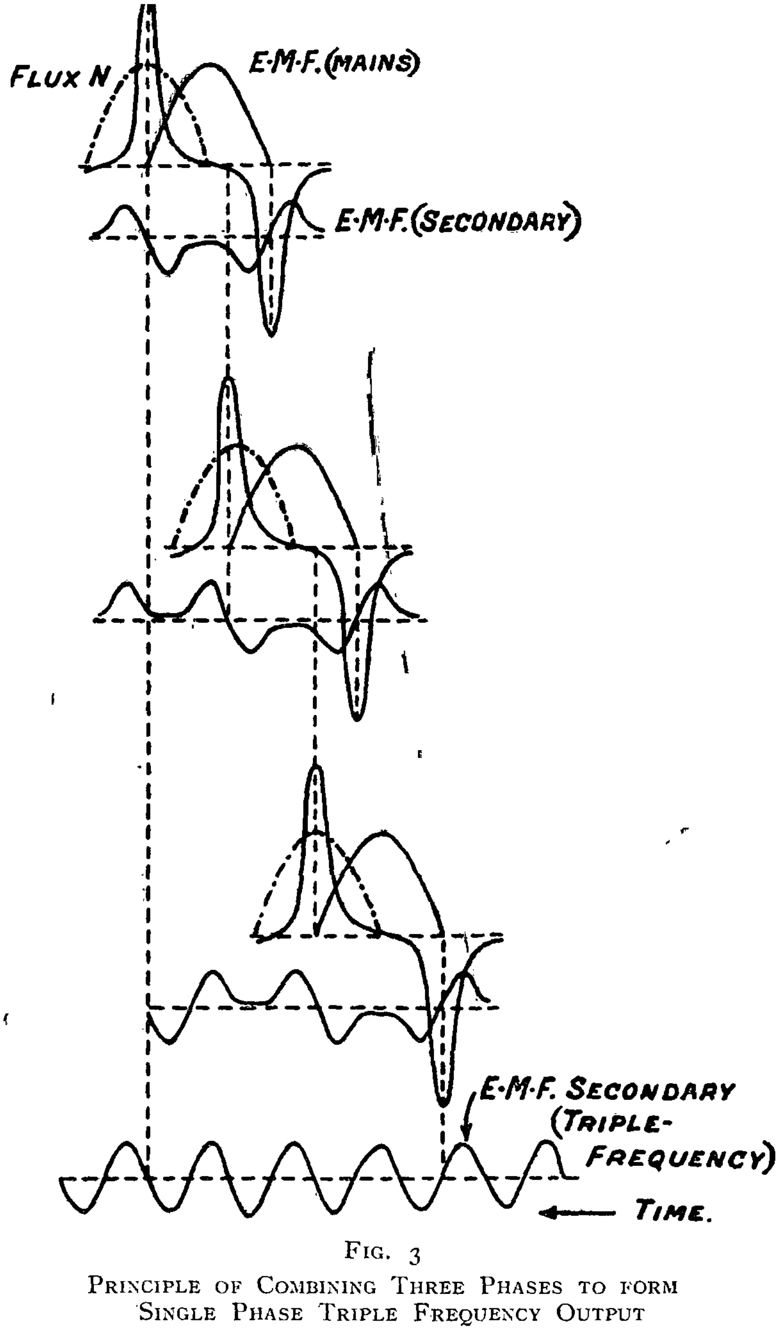
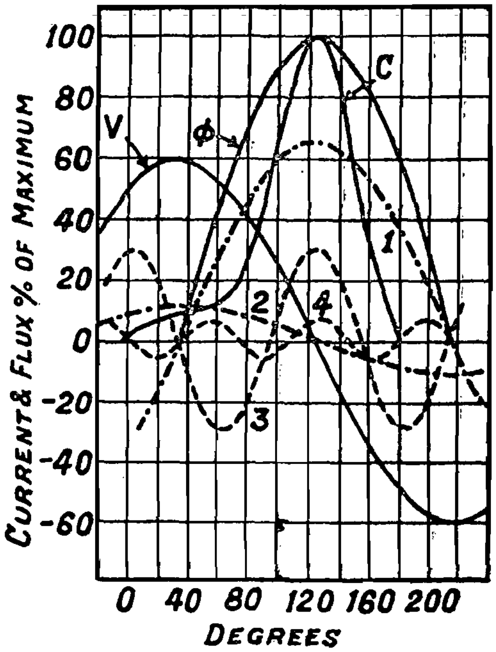


FIG. 3

PRINCIPLE OF COMBINING THREE PHASES TO FORM SINGLE PHASE TRIPLE FREQUENCY OUTPUT (TAYLOR'S ARRANGEMENT)

Effect of distortion of choke coil P.D.—The essential part of the action of the tripler has been dealt with above. It is, however, further



MAX. COMPLEX WAVE = 100

$R_1 = 65{\cdot}0$ $\phi_1 = 27°1'$
$R_3 = 29{\cdot}9$ $\phi_3 = 73°55'$
$R_5 = 6{\cdot}2$ $\phi_5 = 170°24'$

FIG. 6

complicated by the fact that, if the supply P.D. is sinusoidal the choke coil P. D. becomes distorted and contains a triple harmonic component equal and in phase-opposition to the triple harmonic P.D. across the transformer. The exciting current wave therefore differs from those shown in figs. 5 and 6, as explained below.

With a sinusoidal P.D. wave the magnetising current of the choke coil contains a third harmonic component of definite magnitude. If, however, a suitable third harmonic component is present in its P.D. wave, the third harmonic component of the magnetising current is correspondingly reduced. If this third harmonic component in the P.D. wave is gradually increased, the third harmonic component of the current will steadily fall until, when the former has attained a certain value, the third harmonic component of the current will vanish and the current wave will become sinusoidal.

Effect of number of turns of transformer.—Assuming that the magnetic circuit of the transformer remains constant and the number of turns of its primary is varied, the choke coil P.D. and the exciting current will vary in the following manner when the supply P.D. is maintained constant. With a very small number of turns in the transformer its induced E.M.F. is small and the shape of the choke coil P.D. will be very nearly similar to that of the supply voltage. If this latter is sinusoidal the current will contain a large third harmonic component. If the number of turns of the transformer is now increased its induced E.M.F. will increase, the choke coil P.D. will become more distorted and the third harmonic component of the current will decrease. This decrease will, however, be smaller than the increase in the number of turns. Consequently, as the number of turns of the transformer is increased, its E.M.F. will rise, the choke coil P.D. will become more distorted and the current wave will contain a decreasing triple-harmonic component until with a very large number of turns it will have a very small amplitude. However, since the product of the triple harmonic component of the current and the number of turns of the transformer will increase, the transformer will become more and more saturated. It will thus be seen that under suitable conditions it is possible for the tripler to function satisfactorily as a frequency changer even with extremely small triple-frequency component in its current.

Effect of saturation of the transformer.—The effect of saturation of the transformer is as follows. Since the fundamental and fifth harmonic components of the M.M.F. in the transformer vanish, its magnetism is determined solely by the third harmonic component of the current and its multiples which are very small. The M.M.F. wave can therefore be regarded as a more or less sinusoidal triple harmonic wave. At low values of induction the flux and the induced E.M.F. waves will be nearly sinusoidal, but as the core becomes more and more saturated, the flux and the E.M.F. waves will become more and more distorted.

Tripler on load.—When a load is connected across the secondary of the transformer it gives rise to third harmonic ampere-turns in the secondary. In order to neutralise these, equal and opposite ampere-turns are called into play in the primaries and the third harmonic component of the tripler current increases. As this current is the magnetising current of the choke coil, the choke coil P.D. becomes more sinusoidal. Since the transformer P.D. is the difference between the choke coil P.D. and the sinusoidal impressed P.D., its magnitude decreases as the choke coil P.D. becomes more sinusoidal. Consequently, as the load increases, the current contains an increasing triple-harmonic component, the choke coil P.D. becomes more sinusoidal and the transformer P.D. decreases until, on short-circuit, the current

contains a large triple-harmonic component, the choke coil P.D. becomes practically sinusoidal and the transformer P. D. vanishes except for the small internal drop due to its impedance. The tripler then behaves as if the transformer were not present and the choke coils connected directly across the supply mains.

EXPERIMENTAL.

The following experiments serve to illustrate the action of the frequency tripler more fully.

Delta Connection

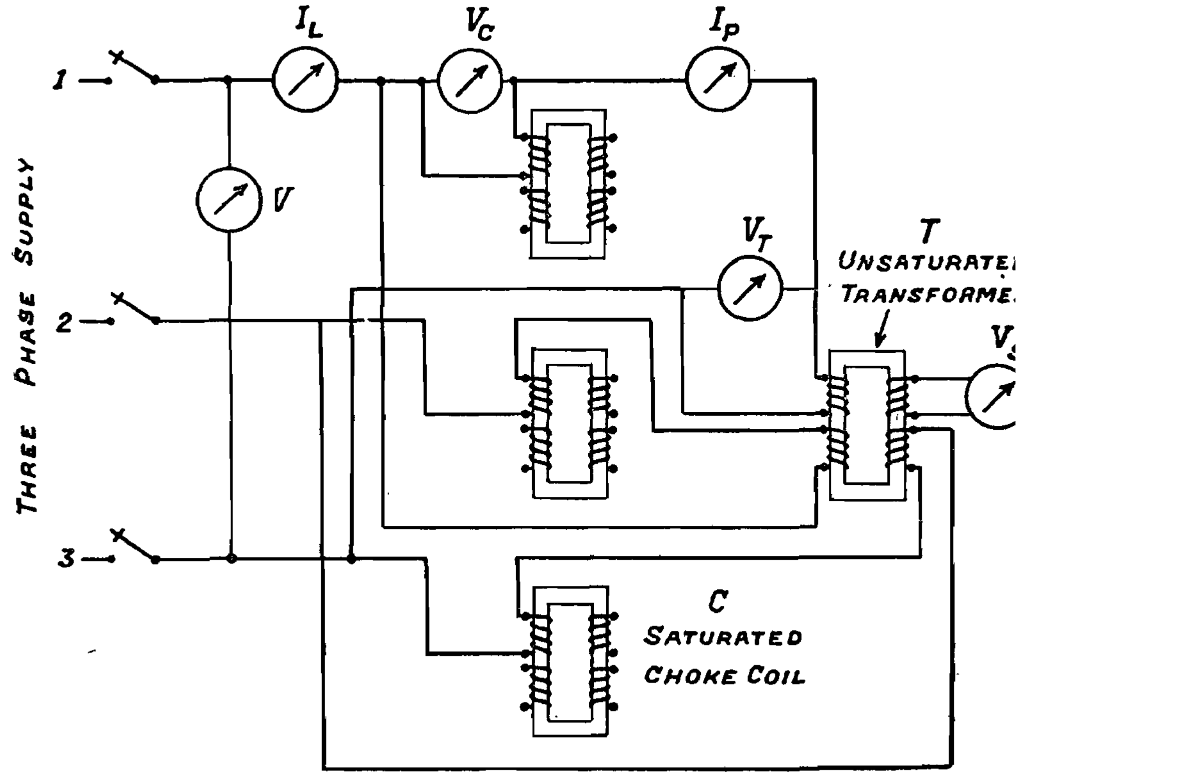


Fig. 7
Diagram of Delta Connection

Fig. 7 shows the diagram of connections of the tripler which was supplied at 25 cycles from a 25/60 cycle, three-phase, 92/220 volt, 12.5/30 kW., sine-wave generator. Four similar 100/200 volt, 25 cycle, 10 kVA., core type, single-phase transformers each having

four separate windings on the primary side were used as choke coils C and also as transformer T. In the case of the choke coils only one of the four primary windings was used, while in the case of the transformer T, one coil was connected to each phase and the fourth coil was used as the secondary.

The results of the experiments are shown in figs. 8 and 9 which correspond to no load condition with impressed P.D.'s of 17 and 37

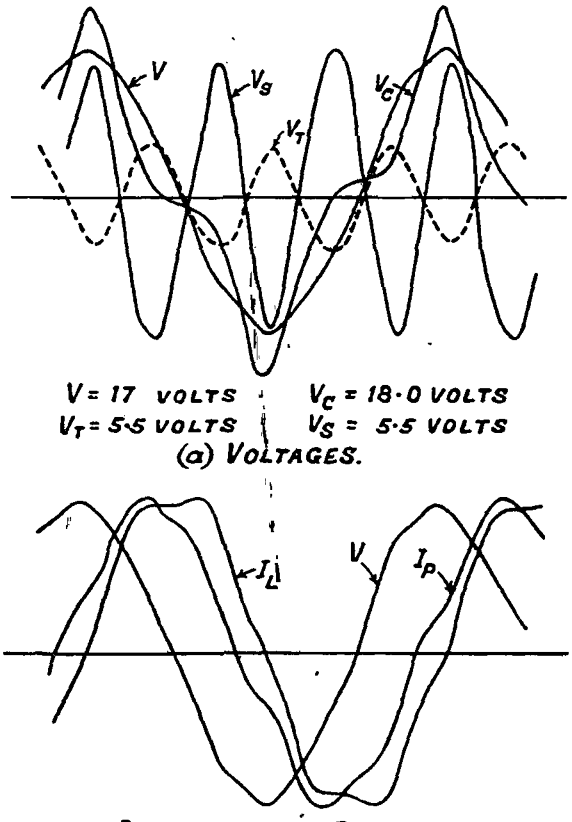


Fig. 8

Delta Connection

volts, respectively. As stated above the E.M.F., V_S induced in the secondary of the transformer is a triple-frequency wave due to the resultant triple-frequency flux in the transformer. Similarly the voltage, V_T across each of the other three windings of the transformer is a triple-frequency wave since it is also caused by the same flux. The slight impurity of this wave is due to leakage in the transformer, particularly at the higher voltage. It must be noted that V_S and V_T are

al in magnitude but drawn to different scales. The voltage V_C oss the choke coil is the difference between V and V_T and thus tains a considerable third harmonic component.

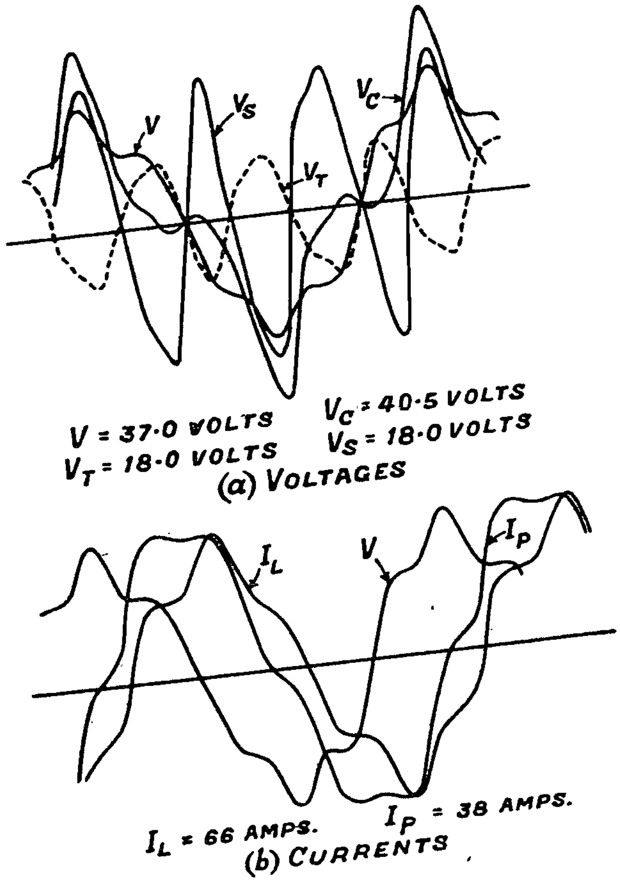


FIG. 9
DELTA CONNECTION

The phase current I_P is a complex wave containing the funda-mental, the third and the fifth harmonic components. The line curren I_L is the vector difference of currents in phases 1 and 2, which hav a phase difference of 120°. It therefore contains only the fundament and the fifth harmonic, the third harmonics of the two phases bein neutralised by each other.

It will be seen that the impressed P.D. wave, V in fig. 9 (a) distorted and contains a prominent fifth harmonic. This is no dou ...rmature reaction of the fifth harmonic component of I_L

Star Connection—Neutral Isolated

When the frequency tripler was star-connected as shown in fig. 10 and the neutral not connected to the neutral of the generator, the results shown in fig. 11 were obtained.

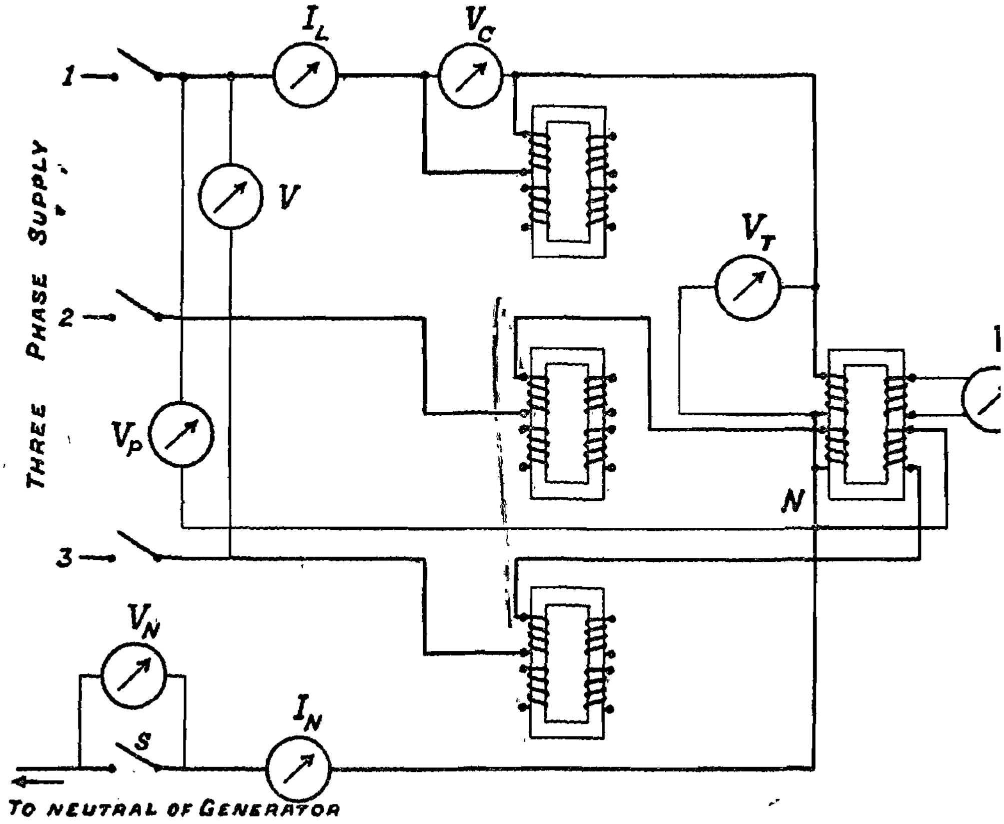
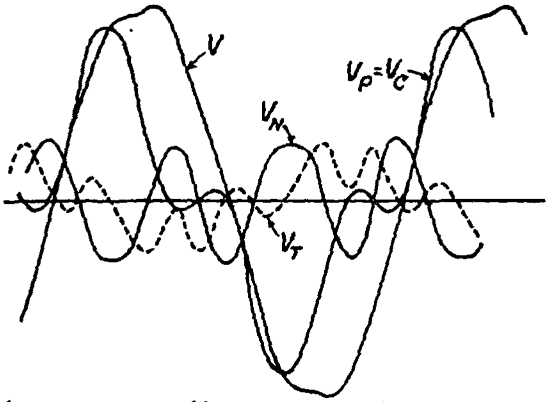


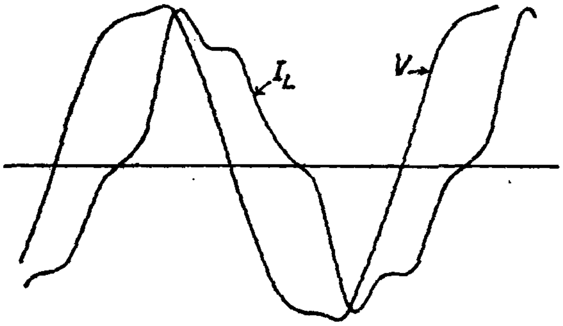
Fig. 10
Diagram of Star Connection

As the neutral is isolated from the neutral of the generator, there is no path for the flow of the triple-frequency component of the exciting current. Consequently the phase voltage becomes distorted and contains a strong third harmonic. The current wave contains the fundamental and the fifth harmonic together with feebler higher harmonics. As it does not contain a third harmonic and as the fundamental and the fifth harmonic of M.M.F. in the transformer are neutralised by the joint action of the three phases, there is no resultant flux in the transformer and consequently no E.M.F. is induced in its windings.

The small P.D., V_T across it is due to the resistance drop and asymmetry of the transformer. The whole arrangement behaves as if the transformer were not present and the choke coils star-connected across the supply mains. As the phase voltage contains the third harmonic,



$V = 61{\cdot}75$ VOLTS. $V_C = 40{\cdot}25$ VOLTS. $V_P = 40{\cdot}25$ VOLTS
$V_N = 17{\cdot}50$ VOLTS $V_T < 1{\cdot}00$ VOLTS
(a) VOLTAGES



$I_L = 39$ AMPS.
(b) CURRENT

FIG. 11
STAR CONNECTION—NEUTRAL ISOLATED

the neutral is unstable and there is a triple-frequency P.D. between it and the neutral of the generator as indicated by V_N. This connection is therefore incapable of functioning as a frequency changer.

In this experiment the supply was obtained from a 25 cycle, 3 phase, 100 volt, 20 kVA. generator with accessible neutral.

Star Connection—Neutral Connected

If the neutral is now connected to the neutral of the generator by closing the switch S, the whole action is transformed, the arrangement behaves like the delta connection and functions again as a frequency tripler, as shown in fig. 12.

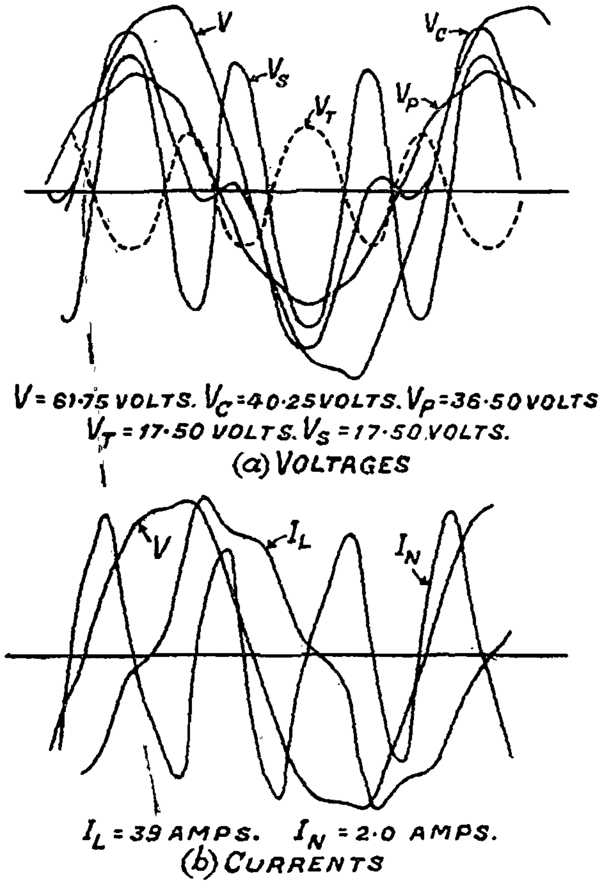


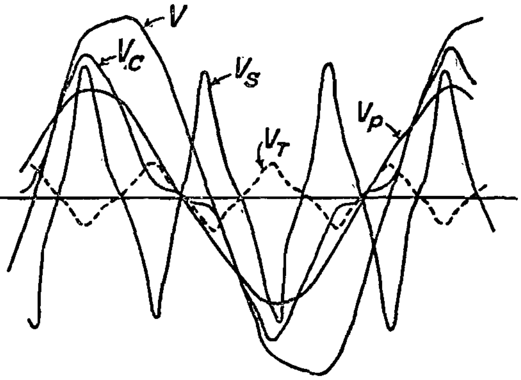
Fig. 12
Star Connection—Neutral Connected
(22 Turns in Transformer)

A path is now provided for the third harmonic component of the exciting current and this flows through each phase, down the neutral, to the neutral of the generator, as shown by I_N in fig. 10. This third harmonic component gives rise to a third harmonic flux in the transformer as in the case of delta connection, and thus induces a triple frequency E.M.F. in each of its windings. The neutral, being directly connected to the neutral of the generator, is stabilised and the

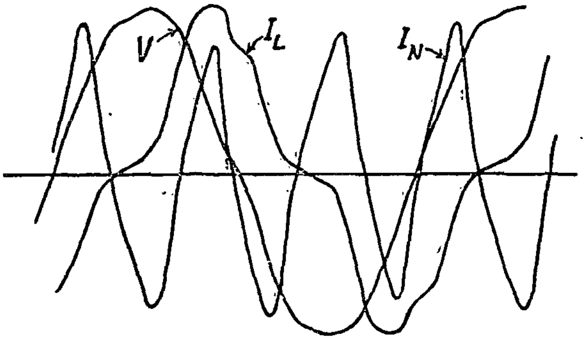
phase P.D., V_P is reduced to a sine wave except for a slight distortion due to armature reaction in the supply generator. As the transformer P.D. is a triple-frequency wave, the choke coil P.D. becomes distorted and contains a third harmonic.

The line current contains the third and the fifth harmonic in addition to the fundamental. The presence of the fifth harmonic is apparent from the current wave I_L, while the amplitude of the third harmonic component is obviously one third of that of the neutral current I_N.

Effect of number of turns of the transformer.—Since the neutral current is proportional to the third harmonic component, this connection affords a very easy means of investigating the relation between this component and the number of turns of the transformer. Fig. 13 shows



$V = 60{\cdot}0$ VOLTS. $V_C = 40{\cdot}5$ VOLTS. $V_P = 38{\cdot}5$ VOLTS.
$V_T = 9{\cdot}5$ VOLTS. $V_S = 56{\cdot}0$ VOLTS.
(a) VOLTAGES



$I_L = 54$ AMPS. $I_N = 37{\cdot}5$ AMPS.
(b) CURRENTS

FIG. 13
STAR CONNECTION—NEUTRAL CONNECTED
(4 TURNS IN TRANSFORMER)

the curves obtained when each of the transformer primaries was provided with four turns. These can be compared with those shown in fig. 12 which were obtained with 22 turns in the primaries of the transformer. The neutral current has increased from 2 amps to 37·5 amps; the wave shape of current I_L has been considerably modified by the presence of the strong third harmonic; the secondary voltage of the transformer contains the ninth harmonic due to saturation of the transformer; and the choke coil P.D. contains a visible ninth harmonic due to the presence of a strong ninth harmonic in the transformer P.D. It may be mentioned that intermediate values were obtained with 8 and 12 turns in the primaries of the transformer. These experiments clearly show that under suitable conditions the third harmonic component of the current may be very small without affecting the operation of the tripler.

Effect of leakage.—If the three primary windings of the transformer are arranged on the three limbs and the secondary on the fourth

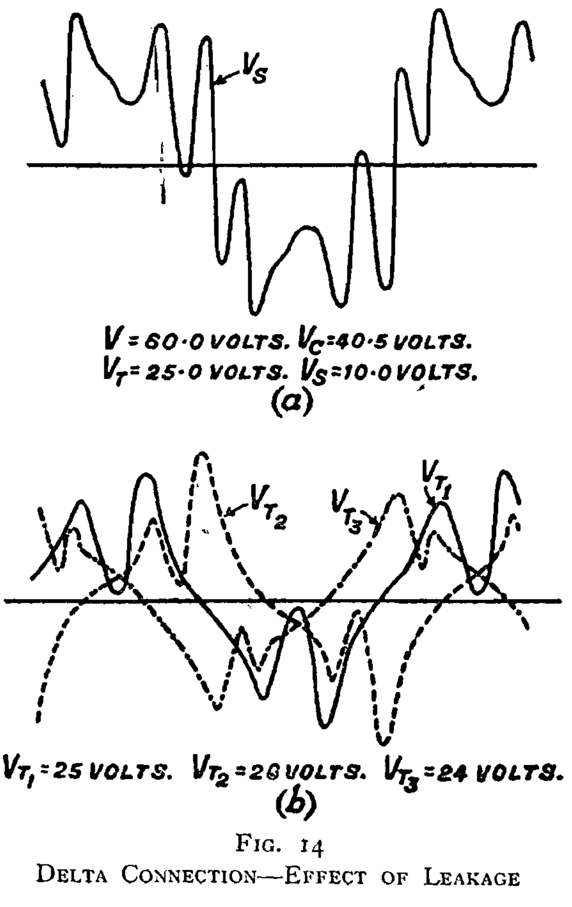


FIG. 14
DELTA CONNECTION—EFFECT OF LEAKAGE

limb of an Epstein Tester and if the cross section of its core is small so as to become easily saturated, there would be considerable leakage of flux between the ends of each limb. Consequently, the fundamental and fifth harmonic components of the fluxes due to the three-phase primary currents would not be completely neutralised and the resultant flux through the secondary would contain these components in addition to the third harmonic. The wave of E.M.F. induced in the secondary would therefore not be a pure triple harmonic wave, but be highly distorted due to the presence of the fundamental and the fifth harmonic. Fig. 14 (a) shows such a wave obtained when the transformer in fig. 7 was replaced by an Epstein Tester and a large P.D. (60 volts) impressed on the tripler. Fig. 14 (b) represents the

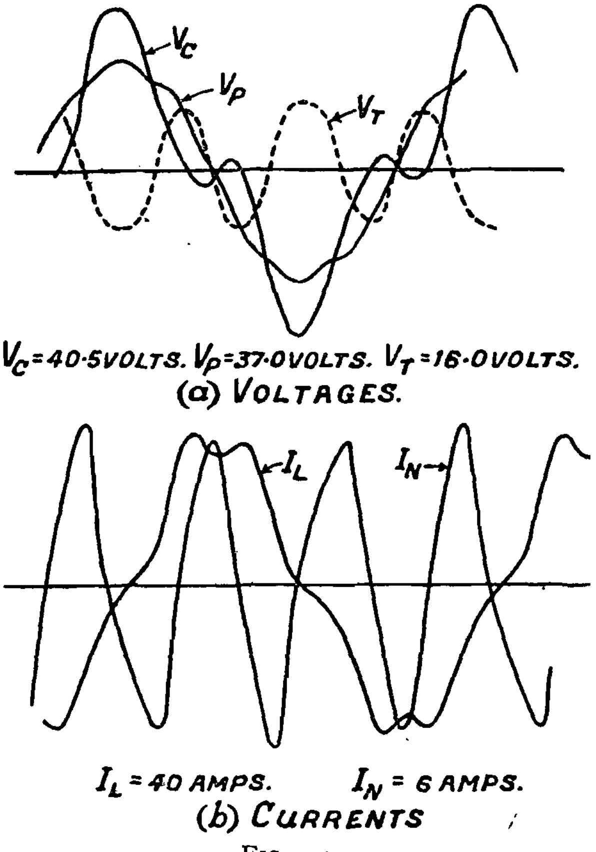


FIG. 15

STAR CONNECTION—NEUTRAL CONNECTED

(12 TURNS; OPEN-CIRCUIT CONDITIONS)

E.M.F.'s induced in the primary windings and indicates that the fluxes in the cores surrounded by these windings contain the fundamental,

third and fifth harmonics. Consequently, the secondary E.M.F. shown in fig. 14 (a) can be regarded as resulting from a combination of these. Experiment at a much lower impressed voltage gave a more or less pure triple harmonic secondary E.M.F., because in that case the leakage was small due to the low value of induction in the Epstein Tester.

Effect of load.—Figs. 15 and 16 shows respectively the open and short circuit conditions in the tripler with 12 turns in each winding of

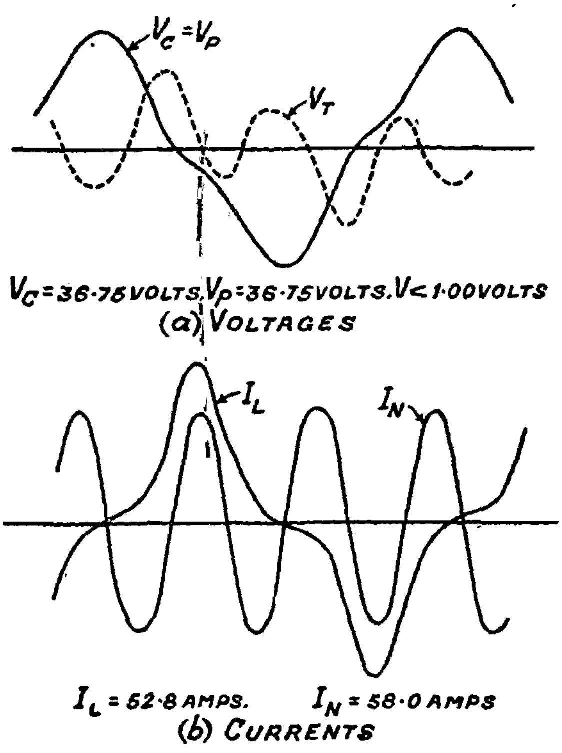


FIG. 16

STAR CONNECTION—NEUTRAL CONNECTED

(12 TURNS; SHORT-CIRCUIT CONDITIONS)

the transformer. It will be seen that the choke coil P.D., V_C is considerably purer on short-circuit than on open-circuit, its slight distortion in the former case being due to the impurity of the supply P.D. The transformer P.D. has dropped from 16 volts to a very small value and the triple harmonic component of the main current represented by I_N, has increased from 6.0 amps. to 58.0 amperes.

The following table shows the change in the magnitudes of the various quantities with a constant impressed P.D. as the load current is increased :—

Transformer secondary current (amps)	Transformer secondary P.D. (volts)	Third harmonic component of current (amps)	Total current (amps)
0.0	31.0	2.0	45.0
5.0	26.5	5.5	45.25
10.0	23.5	7.75	45.5
15.0	20.0	10.25	46.5
20.0	15.5	13.0	48.0
25.0	11.0	15.50	50.0
30.0	5.0	18.25	52.0
32.5	0.0	19.3	52.8

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